The vascular responses of the spleen to intravenous infusions of catecholamines, angiotensin and vasopressin in the anaesthetized cat

C. V. GREENWAY AND R. D. STARK

Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada

Summary

1. Splenic arterial flow and splenic weight were recorded in cats anaesthetized with sodium pentobarbitone. The responses of the spleen to catecholamines, angiotensin and vasopressin were investigated.

2. Catecholamines caused responses mediated by α- and β-adrenoceptors in the arteriolar smooth muscle, but only insignificant β-adrenoceptor responses could be elicited from the capsular smooth muscle. The difficulties in elucidating the mechanism of action of catecholamines on arteriolar smooth muscle are discussed.

3. Angiotensin caused marked vasoconstriction, but contraction of the capsular smooth muscle was less marked. Vasopressin caused vasoconstriction but had no effect on capsular smooth muscle. Thus these peptides constrict the resistance vessels but produce much weaker contraction of the capsule.

4. These responses are discussed in relation to the splenic responses to acute haemorrhage.

Introduction

Understanding of the vascular responses of the spleen to complex stimuli such as haemorrhage requires a knowledge of the responses to the various influences acting after blood loss. Adrenaline and noradrenaline (Manger, Bollman, Maher & Berkson, 1957; Glaviano, Bass & Nykiel, 1960), angiotensin (Regoli & Vane, 1966; Hodge, Lowe & Vane, 1966) and vasopressin (Beleslin, Bisset, Haldar & Polak, 1967; Clark & Rocha e Silva, 1967) are present in the blood after haemorrhage. In a previous paper (Greenway & Stark, 1969) we investigated the mechanisms of the splenic responses to haemorrhage. The splenic contraction was caused only by activity of the splenic nerves and adrenal medullae. The arteriolar vasoconstriction was due to activity of the splenic nerves and adrenal medullae, and the actions of angiotensin and some other vasoconstrictor substance, possibly vasopressin. According to these findings, catecholamines, angiotensin and vasopressin should constrict the splenic resistance vessels, but there should be no reduction in splenic weight during infusions of angiotensin and vasopressin in the quantities present after a haemorrhage.

To test this prediction we studied the actions of intravenous infusions of catecholamines, angiotensin and vasopressin on splenic weight and blood flow. In addition, some of the interrelationships between the responses mediated by α- and β-adrenoceptors in the spleen were investigated. Previous studies by Ottis, Davis & Green

(1957) and by Davies, Gamble & Withrington (1968a, b) involved cannulation of the arterial supply which may disturb vascular responsiveness (Folkow, 1953), while Ross (1967) dealt only with flow changes. We therefore measured splenic flow and weight simultaneously, and the methods did not involve cannulation of the splenic artery (Greenway, Lawson & Stark, 1968).

Methods

Thirty-eight cats, weighing 2–4 kg, were anaesthetized by intraperitoneal injection of sodium pentobarbitone (30 mg/kg, Abbott). Additional doses of 8 mg pento-barbitone were given through a cannula in a forelimb vein when reflex ear or limb movements returned. Details of the method used to record splenic and femoral arterial pressures and splenic flow and weight have been described (Greenway *et al.*, 1968). Briefly, splenic arterial blood flow was measured by an electromagnetic flowmeter (Nycotron) while the spleen lay on a cradle suspended from a strain gauge transducer. A micrometer-controlled artery clamp could be tightened to prevent an increase in splenic arterial pressure when systemic arterial pressure increased. The splenic nerves were divided ; in four experiments the nerves were inserted into a bipolar ring electrode to permit nerve stimulation. The control values for flow, weight and arterial pressure lay within the ranges previously described (Greenway *et al.*, 1968).

Stock solutions of (−)-noradrenaline tartrate (1 mg base/ml distilled water, British Drug Houses), (−)-adrenaline (1 mg base/ml 0·01 N HCl, British Drug Houses) and isoprenaline HCl (1 mg base/ml distilled water, British Drug Houses) were diluted in 0·9% sodium chloride solution containing ascorbic acid (0·2 mg/ml). All doses are expressed in terms of free base. Angiotensin (Hyper-tensin, Ciba Laboratories Ltd.) and lysine vasopressin (Sandoz Products Ltd.) were dissolved in 0·9% sodium chloride solution. The drugs were infused intravenously by a motor driven syringe. The doses were not varied with the body weight of the cats, but mean body weight values are given in the **Results** section. In seven cats, isoprenaline was infused into the splenic artery through a cannula in the common trunk of the hepatic artery. Propranolol hydrochloride (1 mg/kg, Inderal, I.C.I.) and phenoxybenzamine (10 mg/kg, Dibenzyline, Smith, Kline & French) were dissolved in 0·9% sodium chloride solution and injected intravenously. A period of 15 min after injection of propranolol and 60 min after injection of phenoxy-benzamine was allowed to elapse before the infusions of catecholamines were repeated.

Drugs were infused for periods of 5 min. The various parameters were measured at 1 min intervals during the infusions and splenic vascular conductance and weight were evaluated. Vascular conductance is flow divided by the arterial minus venous pressure and is thus the reciprocal of vascular resistance. The reasons for using conductance in preference to resistance have been discussed previously (Stark, 1968). The means of the values at 2, 3, 4 and 5 min for both weight and conductance were calculated and were expressed as percentages of the pre-infusion levels.

Results

Isoprenaline

On twenty-four occasions in eleven cats (mean weight 2·8 kg), isoprenaline was infused intravenously at rates between 0·25 and 2 μg/min. The response in a typical

experiment is shown in Fig. 1 and it can be seen that flow increased rapidly. There-after, splenic arterial pressure usually decreased and flow tended to decline. Con-ductance, however, showed a plateau response. The means of all the responses are shown in Fig. 2.

The splenic weight response was usually biphasic (Fig. 1), with an increase which never exceeded 15% of the control weight, followed by a decrease to a level which was never more than 15% below the pre-infusion level. The increase in splenic weight might have been due either to active dilatation of the capsule or to passive distension secondary to arteriolar dilatation. An attempt was made to distinguish between these possibilities. On six occasions in three cats, the clamp on the coeliac artery was used to prevent the increase in flow which occurred during intra-arterial infusions of isoprenaline (0·2 µg/min). Each time, splenic weight increased by an
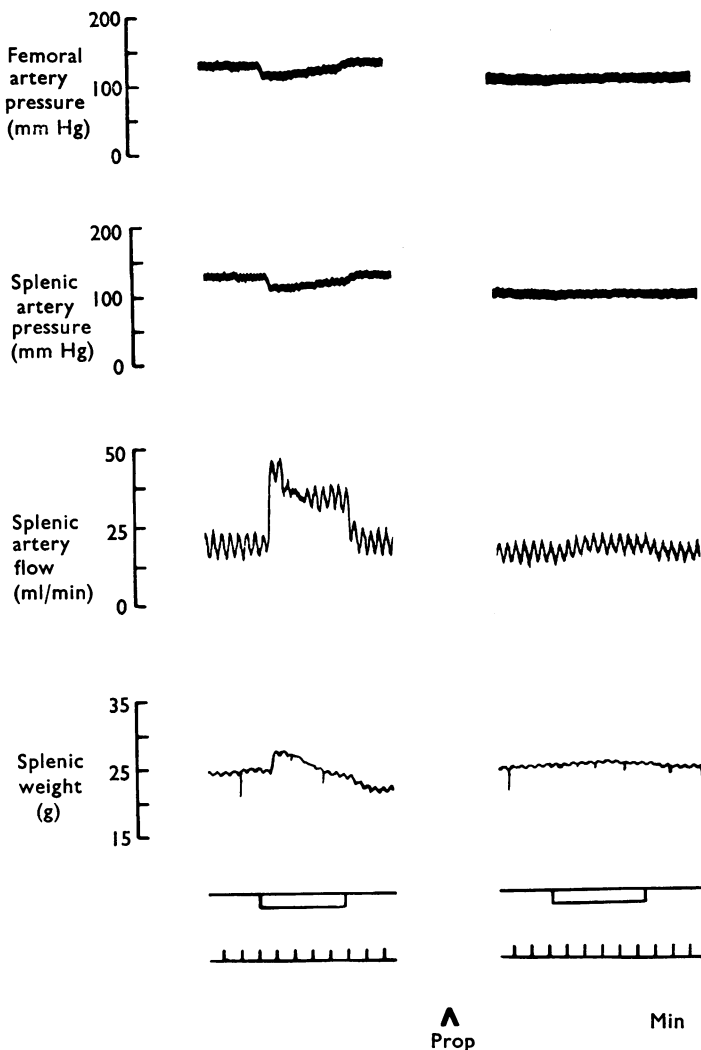

FIG. 1. Cat, 2·4 kg. The responses to intravenous infusions of isoprenaline (1 µg/min) before and after administration of propranolol (Prop, 1 mg/kg).

amount which was similar to that when blood flow was permitted to increase. These experiments suggest that the increase in splenic weight was the result of active dilatation rather than passive distension of the capacitance mechanism.
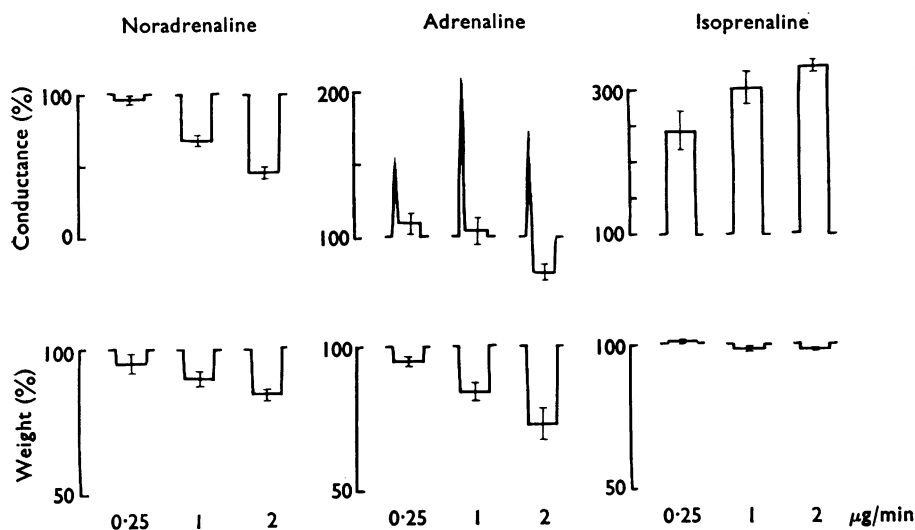

FIG. 2. Mean changes in splenic vascular conductance and weight during infusions of noradrenaline, adrenaline and isoprenaline. Abscissae, rates of infusion of catecholamines (μg/min); ordinates, changes in vascular conductance and weight (% of controls). Vertical bars, S.E. of means.
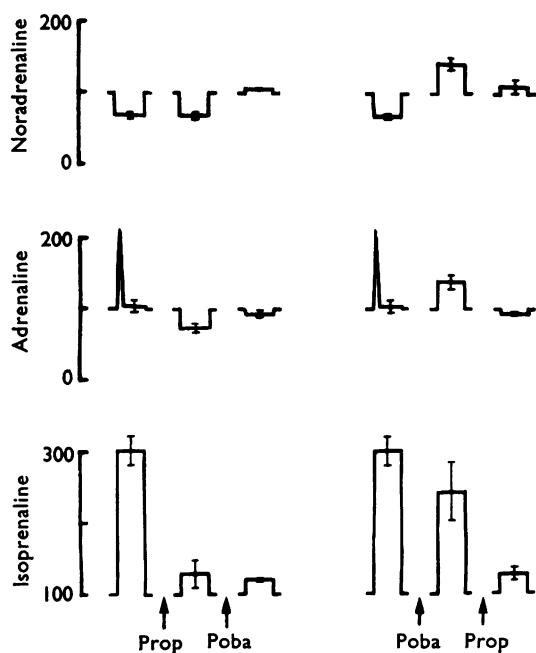

FIG. 3. Mean changes in splenic vascular conductance during infusions of noradrenaline, adrenaline and isoprenaline at the rate of 1 μg/min, and the effects of propranolol (Prop, 1 mg/kg) and phenoxybenzamine (Poba, 10 mg/kg) on the responses. On the left side are shown the effects of propranolol given first and on the right side are shown the effects of phenoxybenzamine given first. Vertical bars, S.E. of means.

After propranolol, the responses to isoprenaline were almost abolished (Figs. 1 and 3), but the responses before and after phenoxybenzamine were similar (Fig. 3).

Noradrenaline

Noradrenaline was infused intravenously on twenty-one occasions in thirteen cats (mean weight 2·7 kg) at rates between 0·25 and 2 μg/min. Femoral arterial pressure increased during the infusions, but splenic arterial pressure was maintained constant using the arterial clamp. Splenic arterial flow decreased (Fig. 2) and the response was well maintained for the duration of the infusion. Splenic weight decreased (Fig. 2) but there was no evidence of fluid exchange. These responses are similar to those obtained during stimulation of the splenic nerves (Fig. 4 ; Greenway *et al.*, 1968).

After administration of propranolol, the splenic conductance (Fig. 3) and weight responses to noradrenaline were unchanged. When noradrenaline was infused after phenoxybenzamine had been given, blood flow increased but there was little change in weight. This vasodilatation was much reduced after propranolol.

Adrenaline

On twenty-three occasions in fifteen cats (mean weight 3·0 kg) adrenaline was infused at rates between 0·25 and 2 μg/min. Splenic arterial flow showed three types of response. The most frequent response (sixteen out of twenty-three infusions) was a brief increase in flow followed by a return towards control level or even below it. On three occasions in two cats a maintained vasodilatation and on four occasions in four cats a maintained vasoconstriction occurred. Splenic weight decreased during infusions of adrenaline and the responses were larger than those during infusions of the same doses of noradrenaline (Fig. 2). The responses were expressed in the same way as those obtained during noradrenaline infusions but, in addition, the flow at the peak of the vasodilatation was measured and the conductance evaluated. The means of all the responses are shown in Fig. 2.

After propranolol, infusions of adrenaline caused only a decrease in conductance which could be abolished by phenoxybenzamine (Fig. 3). After phenoxybenzamine alone, adrenaline caused only an increase in conductance (Fig. 3) and a large decrease in arterial pressure ; the weight response was similar to that which occurred during infusions of isoprenaline. Both the dilatation and the weight response observed after phenoxybenzamine were abolished by propranolol.

Isoprenaline infusions during nerve stimulation

The results obtained so far suggest that the responses of the spleen to adrenaline were due to simultaneous stimulation of α- and β-adrenoceptors. In contrast, the responses to noradrenaline and splenic nerve stimulation (Greenway *et al.*, 1968) were not modified by propranolol. It was important to determine if it was possible to obtain responses mediated by β-adrenoceptors during stimulation of the splenic nerves (see **Discussion**). On ten occasions in four cats, isoprenaline (0·2 μg/min) was infused into the splenic artery while the sympathetic nerves to the spleen were stimulated at 1–4 Hz (15 V, 1 ms duration). When the splenic flow response to nerve stimulation was steady, the isoprenaline infusion was begun. Splenic arterial

flow increased for the duration of the infusion and a maximal vasodilatation of the arterioles could be produced by a large dose of isoprenaline in spite of concomitant nerve stimulation.

Angiotensin

Angiotensin was infused intravenously on sixteen occasions in three female cats (mean weight 2·7 kg). The rates of infusion were between 0·06 and 1 μg/min. Splenic arterial pressure was maintained at a constant level during the infusions.

At low infusion rates, flow decreased but there was little change in splenic weight. At higher rates, the flow response was larger and splenic weight decreased. During many of these infusions there was a brief increase in blood flow to above the pre-infusion level (Greenway & Stark, 1969); this increase always occurred shortly after the initial decrease in flow. The mean values to which splenic conductance and weight decreased during the infusions are shown in Fig. 4. The responses to stimulation of the splenic nerves are included for comparison (Greenway *et al.*, 1968). When similar degrees of vasoconstriction are compared, nerve stimulation caused a larger contraction of the capsule than did infusion of angiotensin.

In three cats, the responses of the capsule to infusions of a range of doses of angiotensin were not significantly different before and after adrenalectomy.

Vasopressin

In seven male cats (mean weight 3·2 kg) vasopressin was infused intravenously at rates of between 2 and 64 m-u./min while splenic arterial pressure was maintained constant. Splenic vasoconstriction occurred even at low doses (Fig. 4) and at rates of 64 m-u./min conductance was reduced to about 25%. At all these infusion rates there was little decrease in splenic weight and the greatest loss observed was 8%. Vasopressin therefore causes vasoconstriction but has a negligible effect on the capsular smooth muscle.
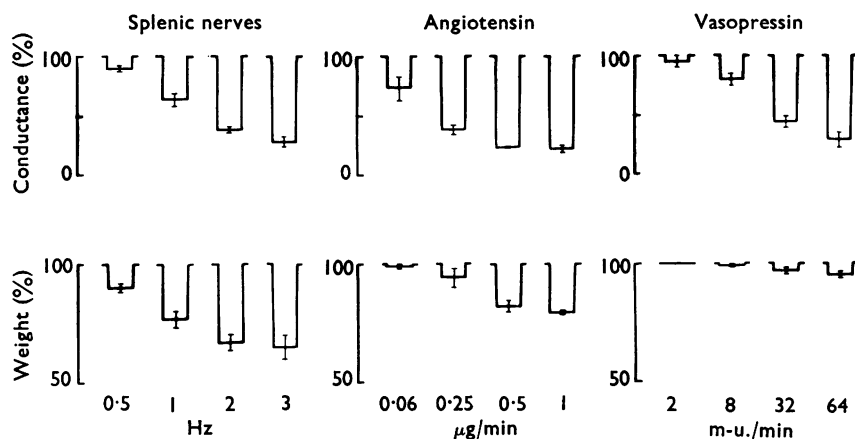

FIG. 4. Mean decreases in splenic vascular conductance and weight during stimulation of the splenic nerves, and infusions of angiotensin and vasopressin. Abscissae, frequency of stimulation or rate of infusion of angiotensin (μg/min) and vasopressin (m-u./min); ordinates, changes in vascular conductance and weight (% of controls). Vertical bars, S.E. of means.

Discussion

The sites of action of catecholamines on the precapillary resistance vessels are not clear. Adrenaline has both α- and β-adrenoceptor actions and the observed responses were balances between these, both in the hepatic (Greenway & Lawson, 1969) and in the splenic vascular beds. Consistent vasoconstrictor responses to adrenaline were observed after propranolol and consistent vasodilator responses after phenoxybenzamine. Isoprenaline produced only β-adrenoceptor responses (Ross, 1967). However, the responses to both injected and neurally released noradrenaline are difficult to interpret. Careful quantitative studies have shown that, in the splenic vascular bed, the response to neurally released (Greenway *et al.*, 1968) or infused (this paper) noradrenaline was not potentiated after propranolol. This was also true in the hepatic vascular bed (Greenway, Lawson & Mellander, 1967). Thus noradrenaline did not appear to cause responses mediated by β-adrenoceptors. There are at least four possible explanations for these findings: noradrenaline cannot stimulate β-adrenoceptors; phenoxybenzamine sensitizes the β-adrenoceptors; β-adrenoceptor responses are completely masked during stimulation of α-adrenoceptors; or noradrenaline does not reach the β-adrenoceptors in effective concentrations. These possibilities will be discussed.

It has been shown that noradrenaline can stimulate β-adrenoceptors in precapillary vascular smooth muscle. After phenoxybenzamine, both neurally released and injected noradrenaline caused vasodilatation in spleen (Greenway *et al.*, 1968; this paper) and in liver (Greenway & Lawson, 1969), although in skeletal muscle injected but not neurally released noradrenaline caused vasodilatation (Glick, Epstein, Wechsler & Braunwald, 1967). However, the failure to obtain reversal in skeletal muscle may have been due to testing the response too soon after the administration of phenoxybenzamine (Greenway & Lawson, 1969) and reversal after dibozane (1,4-*bis*(1,4-benzodioxan-2-yl-methyl) piperazine) has been reported (Viveros, Garlick & Renkin, 1968). Clearly, noradrenaline can stimulate β-adrenoceptors, at least after injection of phenoxybenzamine or dibozane. We cannot excude the possibility, however, that this response is due to sensitization of the vascular β-adrenoceptors (β_2-adrenoceptors, Paton, 1969) by phenoxybenzamine.

Intensive vasoconstriction induced by α-adrenoceptor stimulation might mask any β-adrenoceptor responses, as tentatively suggested by Folkow, Öberg & Rubinstein (1964). However, adrenaline is a potent α-adrenoceptor stimulant and yet the overall response is a balance between stimulation of α- and β-adrenoceptors (see above). Infusions of isoprenaline caused maximal vasodilatation of the splenic arterioles during concomitant stimulation of the sympathetic nerves. This also suggests that α- and β-adrenoceptor responses involve the same smooth muscle cells. If the effects involved smooth muscle of separate resistance sites either in series or in parallel, the maximal vasodilatation elicited by isoprenaline would have been reduced during simultaneous sympathetic nerve stimulation. These findings therefore suggest that α- and β-adrenoceptor responses involve the same smooth muscle and that intense activation of α-adrenoceptors cannot mask a concomitant β-adrenoceptor response.

The fourth possibility is that noradrenaline may not reach the β-adrenoceptor sites. In the case of neurally released noradrenaline, Glick *et al.* (1967) arrived at this conclusion from studies on the vascular bed of skeletal muscle. It is possible that re-uptake of noradrenaline (Brown, 1965) may prevent its diffusion to β-adreno-

ceptor sites. After phenoxybenzamine, the α-adrenoceptors are blocked and the re-uptake of noradrenaline is inhibited (Brown & Gillespie, 1957 ; Thoenen, Hürlimann & Haefely, 1963, 1964 ; Brown, 1965). The transmitter may thus be able to diffuse to β-adrenoceptor sites in effective concentrations and elicit a vaso-dilatation. Inhibition of re-uptake appears to be a slower process than α-adreno-ceptor blockade (Thoenen et al., 1964), and this may account for the fact that it is easier to obtain a reversal at 1 h than at 10 min after injection of phenoxybenzamine (Greenway & Lawson, 1969). However, these arguments are difficult to apply to injected noradrenaline, which presumably diffuses throughout the vessel wall.

Thus we cannot resolve the problem of the site of action of catecholamines on the precapillary resistance vessels without further data. Studies with other inhibitors of noradrenaline uptake might be of value.

The splenic capsule showed α-adrenoceptor responses to noradrenaline and adren-aline, although adrenaline was more potent in this respect than noradrenaline (Ahlquist, Taylor, Rawson & Sydow, 1954). Contraction of the dog spleen in response to noradrenaline, adrenaline and stimulation of the splenic nerves has also been reported (Ottis et al., 1957 ; Davies et al., 1968a, b ; Green, Ottis & Kitchen, 1960). A small biphasic response was obtained during isoprenaline infusion, and during adrenaline infusion after phenoxybenzamine. Both the initial relaxation and the subsequent contraction were abolished by propranolol. Thus in the splenic capsule, stimulation of β-adrenoceptors may cause both relaxation and contraction. This has also been shown in isolated splenic strips (Bickerton, 1963). The mechanism of these opposite responses is not known.

Angiotensin constricted the splenic arterioles but did not contract the perfused spleen of the dog (Boatman & Brody, 1964 ; Davies et al., 1968b). In our experi-ments, there was no significant contraction of the cat spleen at infusion rates of less than 0·5 μg/min, and this is in contrast to the observations of Benelli, Della Bella & Gandini (1964), who reported contraction after intra-arterial injection of 0·005 μg of angiotensin. At intravenous infusion rates of 0·5 and 1 μg/min there was always a splenic contraction, and this was associated with a brief increase in splenic arterial flow at the onset. Feldberg & Lewis (1964) have shown that angiotensin can cause release of adrenaline from the adrenal medullae, and it seemed possible that this was the cause of the contraction of the capsule. However, the responses were not modified by adrenalectomy.

Vasopressin caused dissociation of the responses of arteriolar and capsular smooth muscles in the spleen ; there was a marked decrease in splenic flow, but only a negligible change in splenic weight. Passive effects of the arteriolar constriction on the weight were not expected since complete occlusion of the splenic artery does not cause passive emptying of the spleen (Greenway & Stark, 1969). Vasopressin also causes vasoconstriction in the intestinal vascular bed (Dresel & Wallentin, 1966). The splenic and intestinal vasoconstriction cause a fall in portal pressure which is the basis for the use of vasopressin in the control of bleeding from oeso-phageal varices.

The splenic contraction which follows acute rapid haemorrhage is mediated by the sympathetic nervous system both neurally and hormonally (Greenway & Stark, 1969). The results presented in this paper confirm that both adrenaline and nor-adrenaline in amounts which may be released in the cat (Celander, 1954) contract the splenic capsule. The sympathetic nervous system plays a minor role in causing

·constriction of the splenic resistance vessels, and the results of earlier investigations (Greenway & Stark, 1969) suggest the importance of angiotensin and another vasoconstrictor agent which is probably vasopressin. Vasopressin has been demonstrated in the blood of the cat after haemorrhage (Beleslin *et al.,* 1967 ; Clark & Rocha e Silva, 1967) ; it causes a marked splenic vasoconstriction and has a negligible effect on the capsule. In the dog, angiotensin is formed after haemorrhage at rates of about 0·25–1·5 μg/min (Regoli & Vane, 1966 ; Hodge *et al.,* 1966). No values are available for the cat, but if it is assumed that angiotensin is released in similar amounts per kg body weight, the cat may produce 0·05–0·15 μg/min. Infusions in this dose range produced significant vasoconstriction with little contraction of the capsule.

Thus the splenic responses to catecholamines, angiotensin and vasopressin are consistent with the data in our previous study on the mechanisms of the responses of the spleen to acute haemorrhage.

REFERENCES

AHLQUIST, R. P., TAYLOR, J. P., RAWSON, C. W. & SYDOW, V. L. (1954). Comparative effects of epinephrine and levarterenol in the intact anesthetized dog. *J. Pharmac. exp. Ther.*, **110**, 352–360.

BELESLIN, D., BISSET, G. W., HALDAR, J. & POLAK, R. L. (1967). The release of vasopressin without oxytocin in response to haemorrhage. *Proc. R. Soc. B*, **166**, 443–458.

BENELLI, G., DELLA BELLA, D. & GANDINI, A. (1964). Angiotensin and peripheral sympathetic nerve activity. *Br. J. Pharmac. Chemother.*, **22**, 211–219.

BICKERTON, R. K. (1963). The response of isolated strips of cat spleen to sympathomimetic drugs and their antagonists. *J. Pharmac. exp. Ther.*, **142**, 99–110.

BOATMAN, D. L. & BRODY, M. J. (1964). Analysis of vascular responses in the spleen. *Am. J. Physiol.*, **207**, 155–161.

BROWN, G. L. (1965). The release and fate of the transmitter liberated by adrenergic nerves. *Proc. R. Soc. B*, **162**, 1–19.

BROWN, G. L. & GILLESPIE, J. S. (1957). The output of sympathetic transmitter from the spleen of the cat. *J. Physiol., Lond.*, **138**, 81–102.

CELANDER, O. (1954). The range of control exercised by the "sympathetico-adrenal system". *Acta physiol. scand.*, **32**, suppl. 116, 1–132.

CLARK, B. J. & ROCHA E SILVA, M. (1967). An afferent pathway for the selective release of vasopressin in response to carotid occlusion and haemorrhage in the cat. *J. Physiol., Lond.*, **191**, 529–542.

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**, 42–43P.

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**, 424P.

DRESEL, P. & WALLENTIN, I. (1966). Effects of sympathetic vasoconstrictor fibres, noradrenaline and vasopressin on the intestinal vascular resistance during constant blood flow or blood pressure. *Acta physiol. scand.*, **66**, 427–436.

FELDBERG, W. & LEWIS, G. P. (1964). The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. *J. Physiol., Lond.*, **171**, 98–108.

FOLKOW, B. (1953). A critical study of some methods used in investigations on the blood circulation. *Acta physiol. scand.*, **27**, 118–129.

FOLKOW, B., ÖBERG, B. & RUBINSTEIN, E. H. (1964). A proposed differentiated neuro-effector organization in muscle resistance vessels. *Angiologica*, **1**, 197–208.

GLAVIANO, V. V., BASS, N. & NYKIEL, F. (1960). Adrenal medullary secretion of epinephrine and norepinephrine in dogs subjected to hemorrhagic hypotension. *Circulation Res.*, **8**, 564–571.

GLICK, G., EPSTEIN, S. E., WECHSLER, A. S. & BRAUNWALD, E. (1967). Physiological differences between the effects of neuronally released and bloodborne norepinephrine on beta adrenergic receptors in the arterial bed of the dog. *Circulation Res.*, **21**, 217–227.

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. (1969). Beta-adrenergic receptors in the hepatic arterial bed of the anesthetized cat. *Can. J. Physiol. Pharmac.*, **47**, 415–419.

GREENWAY, C. V., LAWSON, A. E. & MELLANDER, S. (1967). The effects of stimulation of the hepatic nerves, infusions of noradrenaline and occlusion of the carotid arteries on liver blood flow in the anaesthetized cat. *J. Physiol., Lond.*, **192**, 21–41.

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 haemorrhage in the anaesthetized cat. *J. Physiol., Lond.*, **204**, 169–179.

HODGE, R. L., LOWE, R. D. & VANE, J. R. (1966). The effects of alteration of blood-volume on the concentration of circulating angiotensin in anaesthetized dogs. *J. Physiol., Lond.*, **185**, 613–626.

MANGER, W. M., BOLLMAN, J. L., MAHER, F. T. & BERKSON, J. (1957). Plasma concentration of epinephrine and norepinephrine in hemorrhagic and anaphylactic shock. *Am. J. Physiol.*, **190**, 310–316.

OTTIS, K., DAVIS, J. E. & GREEN, H. D. (1957). Effects of adrenergic and cholinergic drugs on splenic inflow and outflow before and during adrenergic blockade. *Am. J. Physiol.*, **189**, 599–604.

PATON, D. M. (1969). The evidence for different types of β-adrenergic receptors. *Am. Heart J.*, **77**, 707–709.

REGOLI, D. & VANE, J. R. (1966). The continuous estimation of angiotensin formed in the circulation of the dog. *J. Physiol., Lond.*, **183**, 513–531.

ROSS, G. (1967). Effects of catecholamines on splenic blood flow in the cat. *Am. J. Physiol.*, **213**, 1079–1083.

STARK, R. D. (1968). Conductance or resistance? *Nature, Lond.*, **217**, 779.

THOENEN, H., HÜRLIMANN, A. & HAEFELY, W. (1963). Removal of infused norepinephrine by the cat's spleen: mechanism of its inhibition by phenoxybenzamine. *Experientia*, **19**, 601–602.

THOENEN, H., HÜRLIMANN, A. & HAEFELY, W. (1964). Dual site of action of phenoxybenzamine in the cat's spleen: blockade of α-adrenergic receptors and inhibition of re-uptake of neurally released norepinephrine. *Experientia*, **20**, 272–273.

VIVEROS, O. H., GARLICK, D. G. & RENKIN, E. M. (1968). Sympathetic beta adrenergic vasodilatation in skeletal muscle of the dog. *Am. J. Physiol.*, **215**, 1218–1225.