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THE PHARMACOLOGY OF ISOLATED VEINS

BY

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In recent years it has become increasingly evident that veins are not just passive tubes for returning blood to the heart, but are in a state of tone whose magnitude affects not only the compliance of the venous " reservoir " but also the resistance to flow in the system (Folkow, 1962; Guyton, 1963). The importance of this system can be gauged from the fact that approximately half of the blood volume lies within the venous system. Therefore a doubling of venous tone could lead to an increase of 25% of the circulating blood volume and, conversely, a reduction of tone could have effects equivalent to a haemorrhage. It therefore would be expected that the action of drugs on the venous system would produce changes in circulatory haemodynamics comparable in magnitude and importance to the action of drugs on precapillary resistance vessels. Despite this, relatively little work has been done on the pharmacology of the venous system either *in situ* or isolated.

The present study concerns the behaviour of isolated venous preparations and their responses to some common vasoactive drugs. A comparison is made of longitudinal and spiral strips and chain preparations obtained from three veins in the rabbit: the external jugular, the posterior caval and the anterior mesenteric. Strips obtained from the anterior mesenteric vein of cats, rabbits and guinea-pigs were also studied.

METHODS

The animals were killed by a blow on the head. A segment of external jugular vein, posterior vena cava (distal to the renal veins) or anterior mesenteric vein (near its proximal end) was removed and immediately placed in oxygenated Krebs-Henseleit solution. The vein was cleaned of adherent tissue and in most experiments spiral or longitudinal strips were cut from the vein by hand. In some experiments rings were cut and three of these were tied together as in a chain. The strips or rings were then suspended under 250 to 500 mg tension in a 10-ml. organ-bath containing Krebs-Henseleit solution maintained at 37° C and bubbled with 95% oxygen and 5% carbon dioxide. When suspended the strips measured 2 to 3 cm by 2 to 3 mm; the preparation consisting of three rings was 2 to 3 cm long, each ring being 2 to 3 mm wide.

Arrangements were such that two vein preparations could be mounted in the bath simultaneously. This allowed comparison of the responses of two types of vein preparations exposed to identical drug concentrations. The responses were recorded either isotonically on a smoked drum with a magnification of five- to six-times or isometrically using an RCA 5734 mechano-electronic transducer and a Leeds-Northrup direct-writing recorder.

The veins were suspended in the bath for at least 30 min before exposing them to drugs. All drugs were dissolved in distilled water and were administered in a volume of 0.1 ml. added to the bath fluid. The doses of agonist drugs were calculated in terms of their respective bases whereas the doses of antagonists were calculated in terms of their salts. The doses are expressed as the final concentrations in the bath.

The following drugs were used: histamine acid phosphate, (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, (±)-isoprenaline sulphate, acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate, vasopressin, angiotensin (Hypertensin, Ciba), atropine sulphate, hexamethonium bromide, dihydroergotamine methanesulphate, phentolamine methanesulphate, mepyramine maleate, antazoline hydrochloride, methysergide (UML 491), pronethalol, cocaine hydrochloride, papaverine sulphate, sodium nitrite and theophylline.

Histological sections of the rabbit posterior vena cava and anterior mesenteric vein were kindly prepared and photographed by Dr G. A. Gresham of the Department of Pathology, Cambridge University.

RESULTS

Spontaneous activity

Preparations from the rabbit vena cava and jugular vein were not spontaneously active, but spontaneous rhythmic contractions were seen in spiral or longitudinal strips cut from the mesenteric veins of all four species (Fig. 1). Usually the rhythm was absent or of small

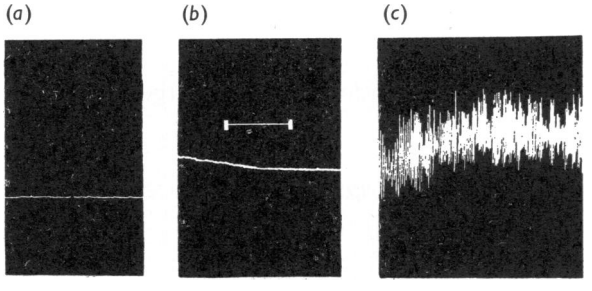

Fig. 1. Isotonic recordings from spiral strips of rabbit external jugular vein (*a*), posterior vena cava (*b*), and anterior mesenteric vein (*c*), 30 min after suspending in an organ-bath. Note the absence of spontaneous activity in (*a*) and (*b*), the loss of tone in (*b*) and the marked spontaneous activity and the increase of tone in (*c*).

amplitude immediately after the preparation was set up, but gradually increased and reached a stable level after about 1 hr and thereafter persisted with little change for up to 6 hr; the basal tone also frequently increased during the first hour. The contractions, which occurred at a frequency of 2 to 6 per minute, corresponded to a change of 10 to 15% in the length of the strip. The rhythmic activity occurred also when the recording was isometric and under these conditions an increase in tension increased both its rate and amplitude. The amplitude was decreased by lowering the temperature and spontaneous activity was usually absent below 32° C.

Rhythmic activity was invariably present in longitudinal and spiral preparations of the anterior mesenteric vein, while ring preparations usually had no rhythm, though occasionally a minimal amount of rhythmic activity was seen. In contrast to longitudinal strips, ring preparations did not gain tone after being suspended in the bath. In histological sections it was seen that mesenteric veins differed from the posterior vena cava in having a well-developed longitudinal muscle coat (Fig. 2). It is presumed that it is the longitudinal muscle which is responsible for the rhythmic behaviour of the mesenteric vein. No effects on the rhythm were produced by atropine, hexamethonium, dihydroergotamine, phentolamine, mepyramine, or cocaine in concentrations up to 10^{-5} g/ml. It thus appears likely that the

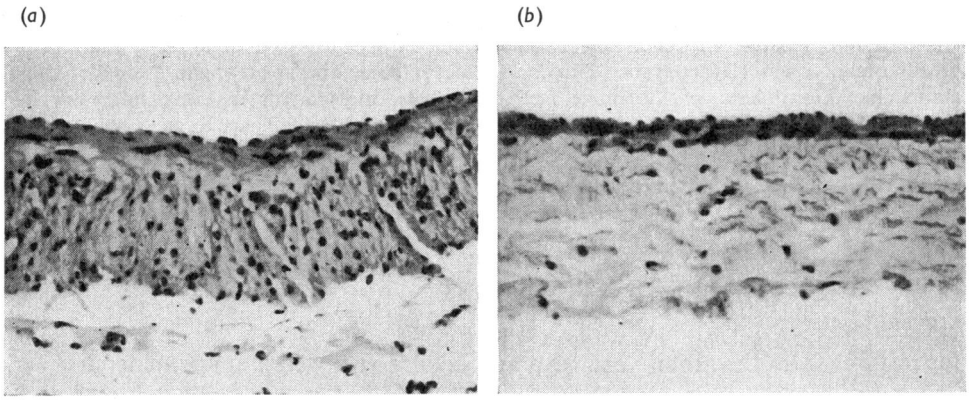

Fig. 2. Photomicrographs (×650) of transverse sections from a rabbit anterior mesenteric vein (*a*) and posterior vena cava (*b*). Haematoxylin and eosin stain. Note the large amount of longitudinal muscle in (*a*) and the almost complete absence of such muscle in (*b*).

rhythm is myogenic. Higher concentrations of dihydroergotamine or cocaine increased the tone without suppressing the rhythm.

Comparison of responses to drugs in strips from rabbit external jugular, posterior caval and anterior mesenteric veins

Histamine. Spiral strips from all three types of vein responded to histamine (Fig. 3). The threshold dose was approximately 10^{-8} g/ml. for posterior caval and mesenteric strips and 10^{-7} g/ml. for external jugular strips. A maximal response was obtained at 10^{-5} g/ml. The rate of spontaneous rhythm in the mesenteric vein was increased by low doses of histamine and the rhythm disappeared only when the tone was considerably increased. Histamine-induced contractions could be blocked by mepyramine and antazoline (10^{-7} g/ml.).

Catechol amines. Noradrenaline produced strong contractions of caval and mesenteric strips. The threshold was about 10^{-9} g/ml. and a maximum response was obtained with 10^{-6} g/ml. The external jugular vein, however, responded poorly to noradrenaline which produced only a small contraction at a concentration of 10^{-5} g/ml. Adrenaline and noradrenaline gave almost identical dose/response curves for both anterior mesenteric and posterior caval preparations (Fig. 4). The responses both to noradrenaline and adrenaline were blocked by phentolamine or dihydroergotamine (10^{-6} g/ml.). Adrenaline did not produce inhibitory responses on the anterior mesenteric vein at any concentration in the range 10^{-11} to 10^{-5} g/ml. even when phentolamine or dihydroergotamine were present in the bathing fluid in concentrations adequate to prevent adrenaline-induced contractions.

Isoprenaline (10^{-9} to 10^{-7} g/ml.) reduced the tone and spontaneous activity of anterior mesenteric strips but contracted the posterior caval strips (Fig. 5). The external jugular preparations were little affected. At a concentration of 10^{-5} g/ml., isoprenaline produced an initial inhibition of spontaneous activity followed by a contraction of the anterior mesenteric vein (Fig. 6). The contractile response to isoprenaline could be inhibited by phentolamine (10^{-6} g/ml.) which did not affect the inhibitory response in the mesenteric

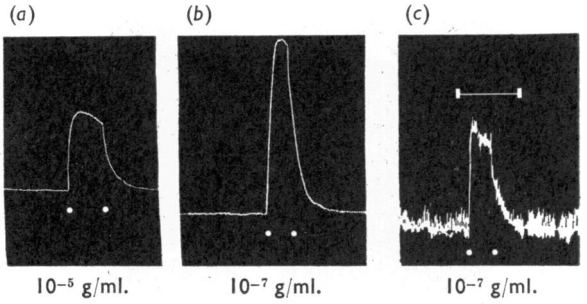

Fig. 3. Isotonic responses to histamine (in concentrations shown) of spiral strips from rabbit external jugular vein (*a*), posterior vena cava (*b*) and anterior mesenteric vein (*c*). In this and similar subsequent Figures the times of adding and washing out of drug are marked by the first and second white dots respectively. Time mark, 10 min.

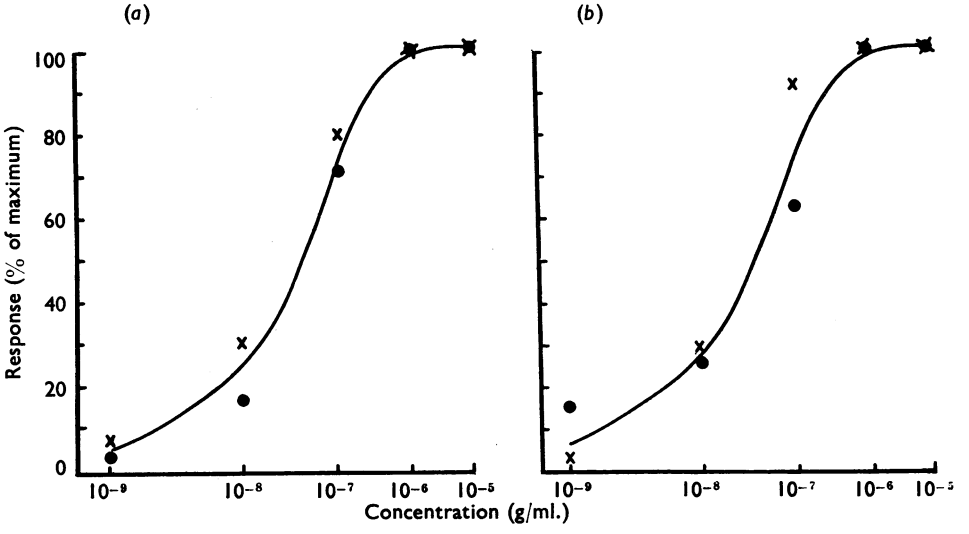

Fig. 4. Dose/response curves to noradrenaline (dots) and adrenaline (crosses) in spiral strips from rabbit posterior vena cava (*a*) and anterior mesenteric vein (*b*).

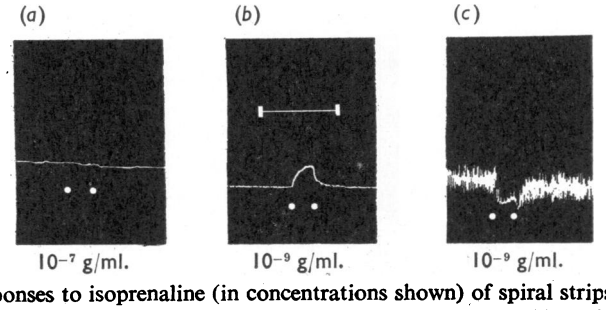

Fig. 5. Isotonic responses to isoprenaline (in concentrations shown) of spiral strips from rabbit external jugular vein (*a*), posterior vena cava (*b*) and anterior mesenteric vein (*c*). The responses shown in (*b*) and (*c*) were recorded simultaneously from two strips suspended in a single tissue bath. Isoprenaline relaxed the anterior mesenteric strip but contracted the posterior vena caval strip. Time mark, 10 min.

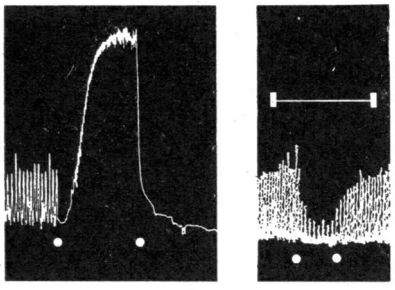

Fig. 6. Isotonic responses of a longitudinal strip of rabbit anterior mesenteric vein to two concentrations of isoprenaline. The high concentration produced an initial inhibition of spontaneous activity followed by a large contraction. The low dose produced inhibition of spontaneous activity without a contraction. Time mark, 10 min.

vein. The latter was, however, suppressed by pronethalol (10^{-6} g/ml.) which also had a slight depressor effect on the contractile response. Pronethalol itself contracted the anterior mesenteric vein strip at a concentration of 10^{-5} g/ml. Tyramine (10^{-5} g/ml.) contracted the mesenteric and caval strips. It was not tested on the jugular strips.

Acetylcholine. Acetylcholine contracted all three types of veins (Fig. 7). The response was poor for the external jugular vein, but the threshold dose was 10^{-7} and 10^{-8} g/ml. for the posterior caval and anterior mesenteric strips respectively. No relaxation was produced by acetylcholine at any dose up to 10^{-5} g/ml. Atropine (10^{-7} g/ml.) blocked and hexamethonium (in doses up to 10^{-5} g/ml.) did not block the response to acetylcholine.

5-Hydroxytryptamine. Of the three types of vein only the anterior mesenteric responded to 5-hydroxytryptamine. The threshold dose was 10^{-8} and a maximal response was obtained at a concentration of 10^{-5} g/ml. The rate of spontaneous contractions tended to be increased by low doses of this drug. Methysergide or morphine, in concentrations up to 10^{-5} g/ml. did not inhibit the contraction produced by 10^{-5} g/ml. of 5-hydroxytryptamine. Methysergide itself contracted the vein at this concentration.

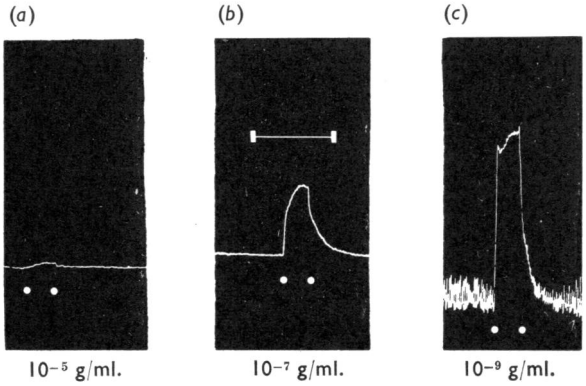

Fig. 7. Isotonic responses to acetylcholine (in concentrations shown) of spiral strips from rabbit external jugular vein (*a*), posterior vena cava (*b*) and anterior mesenteric vein (*c*). Time mark, 10 min.

Polypeptides. Angiotensin contracted external jugular, posterior caval and anterior mesenteric strips, whereas none of the three types of vein responded to vasopressin in doses up to 0.1 U/ml. (Fig. 8). The threshold dose for angiotensin was approximately 10^{-8} g/ml. and a maximal response was obtained at 10^{-5} g/ml. An occasional preparation did not contract to angiotensin at any concentration. Bradykinin (10^{-5} g/ml.) produced a good response in posterior caval and anterior mesenteric strips. It was not tested on external jugular strips. Substance P (10^{-5} U/ml.) contracted the posterior caval strips, the only preparation on which it was tried.

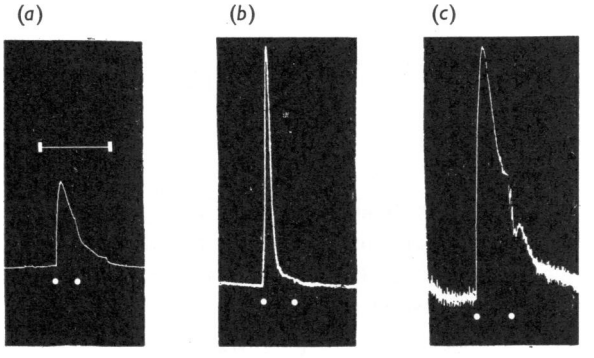

Fig. 8. Isotonic responses to angiotensin (in concentrations shown) of spiral strips from rabbit external jugular vein (*a*), posterior vena cava (*b*) and anterior mesenteric vein (*c*). Note that relaxation occurred while the drug was still present in the bath. Time mark, 10 min.

The response to angiotensin was poorly sustained; relaxation occurred while the drug was still in the bath. Addition of a second dose of angiotensin during relaxation did not produce a response but addition at this time of another agonist such as histamine did produce a contraction.

Smooth muscle relaxant drugs. No drug produced relaxation of either the external jugular or caval strips, but anterior mesenteric vein strips were relaxed by a number of drugs. Apart from isoprenaline (see previously), the most potent of these was papaverine. At a dose of 10^{-5} g/ml. this agent reduced tone and completely inhibited spontaneous activity. Theophylline or sodium nitrite at this concentration relaxed the vein slightly and decreased the amplitude of spontaneous contractions by 10%. Higher concentrations were not tested. The responses of the three types of veins to vasoactive drugs are summarized in Table 1.

Drug responses of longitudinal and circular muscle in rabbit veins

An attempt was made to assess the contributions of longitudinal and circular muscles in the drug-induced contractions of the veins. To this end the responses of a longitudinal strip and a chain preparation from the same rabbit vein were compared. All three types of veins responded to histamine, but the ring preparations of external jugular, caval and mesenteric veins contracted by 25, 50 and 25%, respectively, of their total suspended lengths.

TABLE 1

RESPONSES OF SPIRAL STRIPS FROM THREE TYPES OF RABBIT VEINS TO SOME COMMON VASOACTIVE DRUGS

Drug	Response of External jugular vein	Posterior caval vein	Anterior mesenteric vein
Histamine	Contraction	Contraction	Contraction
Noradrenaline	Contraction (weak)	Contraction	Contraction
Adrenaline	Contraction (weak)	Contraction	Contraction
Isoprenaline (10^{-5} g/ml.)	Nil	Contraction (weak)	Inhibition of spontaneous activity followed by contraction
Isoprenaline (10^{-9} g/ml.)	Nil	Contraction (weak)	Inhibition of spontaneous activity and relaxation
Acetylcholine	Nil	Contraction	Contraction
5-Hydroxytryptamine	Nil	Nil	Contraction
Angiotensin	Contraction	Contraction	Contraction
Vasopressin	Nil	Nil	Nil
Papaverine	Nil	Nil	Inhibition of spontaneous activity and relaxation

In contrast, the longitudinal strips from the same three veins contracted by 4, 7 and 20%, respectively, to histamine. Circular muscle was thus mainly responsible for the contraction of external jugular and caval veins; longitudinal as well as circular muscle played a role in the anterior mesenteric vein response.

With one exception, the specificity of drug response appeared to reside in the type of vein rather than the orientation of muscle. Thus only the anterior mesenteric preparations contracted to 5-hydroxytryptamine and both longitudinal and ring preparations did so. On the other hand, external jugular preparations were rather unresponsive to noradrenaline and this was true of both longitudinal and circular preparations from this vein. Isoprenaline, however, relaxed longitudinal strips but did not relax ring preparations of anterior mesenteric veins. This was the only drug which acted on one type of muscle but not the other type from the same vein.

DISCUSSION

The most striking feature of these results is the difference in behaviour of the three types of veins *in vitro*. Both the presence of spontaneous activity and the pattern of response to drugs appears to depend on the type of vein examined. A recent report (O'Mahoney, 1963) also suggests that the *in vitro* responses of dog veins to drugs varies with the type of vein used.

The anterior mesenteric vein from four different species consistently showed rhythmic spontaneous contractions; the posterior caval and external jugular preparations never showed spontaneous activity. *In vitro* spontaneous contractions of the mesenteric vein were first reported by Franklin (1925) who observed this phenomenon in sheep mesenteric vein rings. However, he observed it only rarely unless sheep serum was added to the bathing fluid. Maloff subsequently reported (1934) that spontaneous activity was consistently observed, even in the absence of serum, in longitudinal strips from cat mesenteric veins. Assuming no species differences, this implied that the longitudinal muscle is involved in producing spontaneous contractions. Our observations that ring preparations of anterior mesenteric vein rarely show spontaneous activity whereas longitudinal strips always do so are additional evidence for this view.

Funaki & Bohr (1964) have recently reported that spiral strips from the portal vein of the rat show spontaneous contractions. It is likely that the vein they used is the same as the one we refer to as the anterior mesenteric vein. The portal vein is formed by the junction of the splenic and anterior mesenteric vein. The vein utilized in the present experiments was distal to the junction with the splenic vein; hence the reason for terming it the anterior mesenteric rather than portal vein.

While the exact mechanism involved in production of the spontaneous activity is not known it appears to be myogenic, since neither cocaine nor any of the common " blocking " drugs inhibit the spontaneous activity. Spontaneous activity can arise from a pacemaker region as in the ureter or from cyclic alterations of membrane potential of many cells as in the gut. The spontaneous contractions in the mesenteric vein are accompanied by spike discharges (Cuthbert & Sutter, 1964), but the mechanism of their initiation remains to be elucidated. The mesenteric vein was also exceptional among those examined in that it possessed tone *in vitro* as demonstrated by the ability of papaverine and nitrite to relax spiral or longitudinal strips of this vein; these substances were without effect on similar preparations of caval or external jugular veins. It has been suggested (Bohr, 1964) that arterial strips do not possess tone because they rapidly lose potassium *in vitro*. Our results suggest that presence or absence of tone in veins is an inherent property of the vessel involved and is not due to phenomena which are peculiar to *in vitro* conditions.

The chain preparations of anterior mesenteric vein did not gain tone on being suspended and isoprenaline did not relax them; longitudinal preparations of this vein frequently gained tone in the bath and were relaxed by isoprenaline. This suggests that much of the inherent tone resides in longitudinal muscle and it is only when this type of muscle is present that tone is present *in vitro*.

It is of considerable interest that raising the resting tension under isometric conditions increased the strength of spontaneous contractions of the anterior mesenteric vein. Assuming that this holds *in vivo*, it is possible that the vein might contract in response to alterations of intraluminal pressure, which would constitute an autoregulatory process.

All the veins that we examined were able to respond to a number of different drugs, but the pattern of response varied with the type of vein. All three types of vein, external jugular, caval and mesenteric, were contracted by histamine and angiotensin. However, only the anterior mesenteric vein responded to 5-hydroxytryptamine. Similarly, low doses of isoprenaline relaxed the anterior mesenteric vein but contracted the posterior vena cava. The external jugular vein was remarkable in being relatively unresponsive to catechol amines.

The results obtained with isoprenaline and the inhibitors, pronethalol and phentolamine, suggest that sympathetic β-receptors are present only in the anterior mesenteric vein, which also has α-receptors. The failure of adrenaline to produce inhibition was presumably due to the predominance of α-receptor stimulation at all doses. It is curious, however, that, even in the presence of the α-receptor blocking drugs phentolamine or dihydroergotamine, no inhibitory responses to adrenaline were seen. The responses of the posterior vena cava are consistent with the exclusive presence of α-receptors in this vessel.

Of the polypeptides, the most interesting results were those obtained with vasopressin and angiotensin. In contrast to its contractile effect on arterial strips and ability to constrict small vessels (Sollmann, 1957), vasopressin was without effect on any of the vein prepara-

tions, confirming the observations of O'Mahoney (1963). On the other hand, angiotensin could contract both circular and longitudinal muscle of all the veins; this has also been reported for veins *in situ* (Nickerson & Sutter, 1964; Zimmerman, Abboud & Eckstein, 1964). The rapidity with which the response to angiotensin fades despite the continued presence of the peptide may account for the failure to detect venoconstriction *in situ* under certain circumstances (for example, Folkow, Johansson & Mellander, 1961). No light has been thrown on the mechanism of this phenomenon.

Both the histological and isolated tissue experiments show that a large proportion of the muscle fibres in the mesenteric vein are longitudinally orientated and that very considerable longitudinal shortening is possible. The physiological significance of this is not immediately apparent but it is possible that the longitudinal fibres are arranged in a shallow helix and may allow long segments of the vein to constrict in a co-ordinated fashion. Spontaneous contractions might then act as a pump to assist venous return; or a sustained contraction might act as a sphincter to trap blood in the visceral region.

It seems clear that the *in vitro* behaviour and responses to drugs of vein strips varies with the site of origin of the preparation. The differing responses may involve the presence of longitudinally oriented muscle in certain veins such as the anterior mesenteric. The functional significance of these findings remains to be defined.

SUMMARY

1. Spontaneous rhythmic contractions were present in spiral or longitudinal strips of rabbit, rat, guinea-pig and cat anterior mesenteric veins. These preparations also possessed tone.

2. Spontaneous activity and tone were absent in spiral or longitudinal strips of external jugular or posterior caval veins from these species and were minimal in ring preparations of rabbit anterior mesenteric vein.

3. Spontaneous activity in the anterior mesenteric vein is probably myogenic since it is unaffected by any of the common blocking drugs or by cocaine in concentrations up to 10^{-5} g/ml. Histological examination showed that there was an abundance of longitudinal muscle in the anterior mesenteric vein and that such muscle was lacking in the posterior vena cava. This, together with isolated tissue experiments, suggests that it is the longitudinal muscle which is responsible for the rhythmic behaviour of the mesenteric vein.

4. The response of a venous strip to a particular drug seems to depend on the type of vein from which it is taken. Thus histamine contracted strips from all three types of veins; noradrenaline and adrenaline strongly contracted strips from posterior caval and anterior mesenteric but not external jugular veins; isoprenaline (10^{-7} to 10^{-9} g/ml.) relaxed anterior mesenteric but contracted posterior caval strips; isoprenaline (10^{-5} g/ml.) contracted both anterior mesenteric and posterior caval strips; acetylcholine contracted all three types of strips but the external jugular strip responded poorly; 5-hydroxytryptamine contracted only anterior mesenteric vein preparations; whereas angiotensin contracted all three types of vein strips, vasopressin contracted none; direct-acting smooth muscle relaxants reduced the tone and inhibited spontaneous activity of the anterior mesenteric strips but had no effect on external jugular or posterior caval strips.

5. It appears that veins cannot be viewed as constituting a pharmacologically homogeneous system.

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