J. Pharm. Pharmac., 1973, **25**, 109–118 *Received September* 8, 1972

The action of vasoactive drugs on longitudinal and circular muscle of dog mesenteric vein

W. J. HALL AND P. C. O'CONNOR*

Department of Physiology, University College, Cork, Ireland

The dog anterior mesenteric vein has outer longitudinal and inner circular muscle layers. The response of the vein to drugs was recorded without disrupting the muscle arrangements by combining constant-flow perfusion of a segment with isometric recording of longitudinal tension. Noradrenaline and acetylcholine were potent stimulants. Tachyphylaxis developed to histamine, 5-hydroxytryptamine and angiotensin. Cocaine and guanethidine potentiated the responses to noradrenaline and fluorescent microscopy confirmed the presence of adrenergic nerves in both muscle layers. Isoprenaline partially relaxed the stimulated vein and this action was blocked by propranolol. Evidence was obtained which suggests the existence in the vein of specific receptors for several of the agonists investigated.

Vascular smooth muscle, especially arterial muscle, is usually arranged in a circular fashion. Longitudinal arterial muscle is a rarity. It is generally not encountered in mammalian arteries with the exceptions of the coronary and pulmonary arteries (Furchgott, 1955). Hence, constant-flow perfusion of isolated vascular segments has been used in recent years to study the reactions of arterial muscle to drugs (McGregor, 1965; de la Lande & Rand, 1965; Rogers, Atkinson & Long, 1966). This approach has been less widely adopted in the study of circular venous muscle. As well as circular muscle, longitudinally orientated muscle has been noted in the walls of several veins (Li, 1940; Bloom & Fawcett, 1968). The reactions of the two layers of venous muscle to drugs have been studied separately by making ring, helical and longitudinal preparations (Sutter, 1965; McConnell & Roddie, 1970). Such preparations, by their nature, disrupt the normal relation between the two layers of muscle.

We have examined the responses of both longitudinal and circular venous muscle to drugs by a method intended to avoid disrupting the intermuscle relations, by combining constant-flow perfusion of a segment of dog anterior mesenteric vein with simultaneous isometric recording of longitudinal muscle tension. Changes in perfusion pressure were taken to represent circular muscle activity and changes in tension, longitudinal muscle activity. A similar approach to the investigation of some responses of bovine mesenteric vessels to drugs has been reported by Williamson (1969).

METHODS

Mongrel dogs, 10–20 kg, were electrocuted and a 5–8 cm segment of anterior mesenteric vein removed and placed immediately in oxygenated Tyrode solution. Tributaries were tied off and excess tissue removed before the preparation was set-up. The proximal end of the vein was cannulated and the vein perfused in a direction retrograde to normal flow by a Watson-Marlow constant-flow inducer. The perfusion fluid passed into the bath and was recirculated continuously, thus both walls of the

* Present address: Department Physiology, University of Manchester, U.K.

vein were brought into contact with the fluid. The pulsatile output of the pump was converted by an air cushion into an almost linear flow. The free end of the vein was attached to a Devices isometric transducer to record changes in longitudinal tension. Alterations in perfusion pressure were recorded by a Statham physiological pressure transducer (Model No. P23AA). The output from the transducers was suitably amplified and displayed on a Devices recorder.

The preparation in the Tyrode solution at 37° was allowed 1 h before its reaction to drugs was tested. In this period, the Tyrode fluid was replaced three times. After this the tension on the longitudinal muscle was set at 2 g and the output of the constant-flow inducer was set to give a resting perfusion pressure of about 10 mm Hg.* Preliminary experiments had shown that these conditions gave optimal responses. The volume of fluid in the system was 450ml. The following drugs (added to the tissue bath) were used: acetylcholine chloride (BDH); (−)-noradrenaline bitartrate, serotonin creatinine sulphate (5-HT), 2-Bromo-D-lysergic acid diethylamide bitartrate (Koch-Light); synthetic angiotensin, phentolamine, guanethidine (Ciba); histamine acid phosphate (Alkem Chemicals); vasopressin (Parke-Davies); isoprenaline sulphate (Boots Pure Drug Co.); propranolol hydrochloride (ICI); phenoxybenzamine hydrochloride (Smith, Kline & French); atropine sulphate (Antigen) and cocaine hydrochloride (May & Baker). Fresh solutions were prepared daily, by dilution with Tyrode, from stock solutions and the doses are expressed as the estimated concentrations per ml of Tyrode in the system.

RESULTS

Morphology

The anterior mesenteric vein of the dog contains two well-developed muscle layers with relatively more longitudinal than circular muscle. The outer layer, next to the adventitia, consists of more or less separated bundles of longitudinally arranged fibres. The inner layer consists of circularly orientated more tightly packed fibres. Formol fluorescent microscopy shows noradrenergic nerves in both muscle layers.

Spontaneous activity

After about 30 min, in at least three out of every four experiments, spontaneous activity became evident and persisted for some hours unless the preparation was exposed to vigorous stimulation by drugs. Fig. 1 shows a typical record of this activity. The upper trace shows spontaneous fluctuations of about 2 g in the tension of the longitudinal muscle. The lower trace shows fluctuations of 1 to 2 mm Hg in perfusion pressure. With a more rapid paper speed, these variations in tension and pressure are shown to be almost in phase. The changes in pressure recorded in Fig. 1 may represent passive changes in resistance to flow, due to longitudinal muscle activity. This is unlikely in view of the frequent observation that much larger rises in tension of the longitudinal muscle did occur in response to drugs without any change in pressure (Fig. 6A).

Noradrenaline, adrenaline, acetylcholine, at supramaximal concentrations ($10\,\mu g\ ml^{-1}$) were equipotent in stimulating the longitudinal and circular muscle layers (Table 1). Histamine and 5-hydroxytryptamine were much weaker agonists on the venous smooth muscle.

* 1 mm Hg = 1·333 mbar.

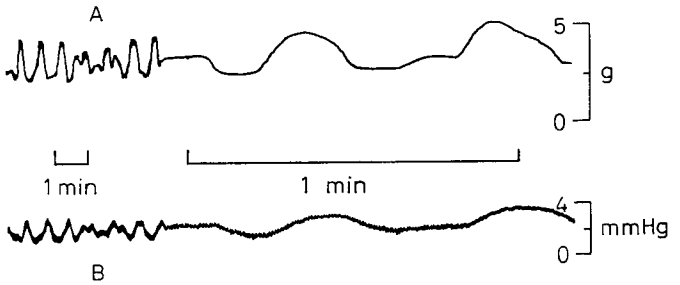

FIG. 1. Spontaneous activity in dog anterior mesenteric vein. Record (a) shows fluctuations in tension of the longitudinal muscle, (b) the changes in pressure (circular muscle) which are almost in phase. Time intervals show one minute. The paper speed was increased, whilst recording, from 1 to 10 cm min^{-1}. In this and other figures the calibration scales represent changes in pressure and tension. The resting pressure and tension levels are *not* indicated by the calibration scales.

Noradrenaline

The cumulative dose response curve to noradrenaline was obtained for ten preparations. The response in each case to graded doses was expressed as a percentage of the maximum response of the vein to the drug. The mean results obtained from the ten experiments are presented graphically in Fig. 2. The longitudinal muscle appears to be more sensitive than the circular muscle to low doses of noradrenaline. The application of Student's *t*-test has shown the responses of the longitudinal muscle to noradrenaline (0·1 to 1 $\mu g\,ml^{-1}$) to be significantly greater than the responses of the circular muscle ($P < 0{\cdot}01$).

Table 1. *Maximum responses of the two muscle layers for various agonists.*

Agonist	Bath concentration $\mu g\ ml^{-1}$	Mean pressure increase ± s.e. mm Hg	Mean tension increase ± s.e. g	No. of experiments
Noradrenaline	10	7·35 ± 2·75	14·69 ± 3·95	36
Adrenaline	10	9·64 ± 3·12	14·06 ± 3·78	11
Acetylcholine	10	6·89 ± 3·92	16·94 ± 3·60	18
Histamine	10	1·27 ± 1·03	4·99 ± 1·32	6
5-Hydroxytryptamine ..	10	1·29 ± 1·14	9·27 ± 3·01	6

Table 2, *Cocaine and guanethidine potentiation expressed as the log dose ratio (Foster 1967).*

	Cocaine*		Guanethidine†	
Experiment	Log dose ratio circular muscle	Log dose ratio longitudinal muscle	Log dose ratio circular muscle	Log dose ratio longitudinal muscle
1	1·0870	1·1206	1·1619	1·2346
2	1·1333	1·4839	1·1304	1·3323
3	1·1223	1·3182	1·1133	1·2409
4	1·3835	1·5904	1·1487	1·1694
5	1·0625	1·5309	1·0577	1·2793

* Five veins were exposed to cocaine 3×10^{-5}M and the responses to noradrenaline of both the longitudinal and circular muscle were potentiated. The degree of potentiation of the longitudinal muscle was significantly greater than the circular muscle ($P < 0{\cdot}05$).

† Five veins were exposed to guanethidine (3×10^{-5}M) for at least 45 min before the effect of guanethidine was tested. Both the longitudinal and circular muscle responses to noradrenaline were potentiated but the degree of potentiation was significantly greater in the longitudinal muscle ($P < 0{\cdot}05$).

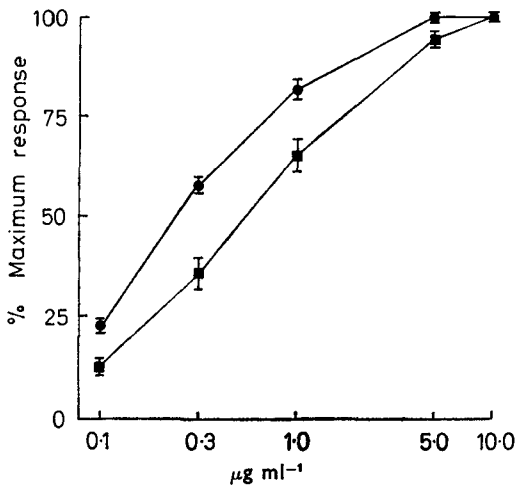

FIG. 2. The (●——●) line shows the mean log dose response curve of the longitudinal muscle to noradrenaline in 10 veins. The (■—■) line shows the mean responses of the circular muscle. Vertical bars indicate s.e. The ordinate shows the observed responses as a percentage of the maximum response. The abscissa shows the concentrations of noradrenaline in the fluid system.

Cocaine

Cocaine is known to potentiate the responses to added noradrenaline of vascular smooth muscle innervated by sympathetic adrenergic nerves (MacMillan, 1959). In the presence of cocaine (3×10^{-5}M) the log-dose response curves for the longitudinal and circular muscle were shifted to the left indicating potentiation (Fig. 3).

In five experiments the degree of potentiation by cocaine was measured as the log dose ratio (Foster, 1967) and the results are presented in Table 2. As can be seen from Table 2, cocaine appears to potentiate the responses of the longitudinal muscle to a greater extent than those of the circular muscle. Application of Student's *t*-test has shown this potentiation of the longitudinal muscle by cocaine to be significantly greater than that of the circular muscle ($P < 0.05$).

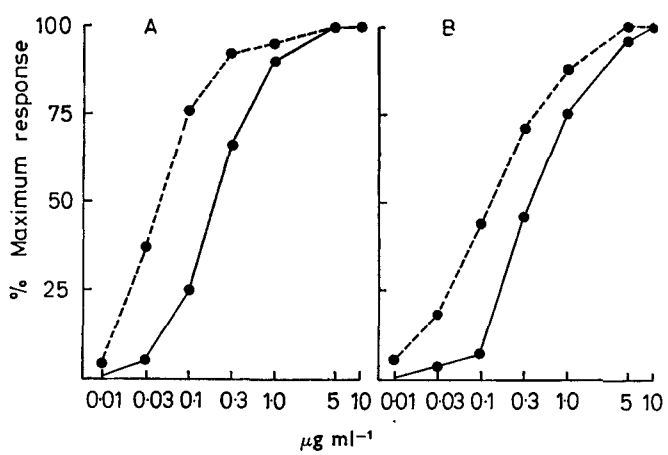

FIG. 3. (a) shows the response of the longitudinal muscle and (b) the circular to noradrenaline (●——●) before and (●- - -●) in the presence of cocaine 3×10^{-5}M.

Guanethidine

Supersensitivity to catecholamines following guanethidine has been demonstrated by a number of investigators including McCubbin, Kaneko & Page (1961), Day & Rand (1961), Boura & Green (1962) and Gokhale, Gulati & Kelkar (1967). In five veins pretreated with guanethidine (3×10^{-5}M) the responses of both the longitudinal and circular muscle to noradrenaline were potentiated (Table 2). As with cocaine, potentiation of the circular muscle response to noradrenaline was significantly less than the longitudinal muscle ($P < 0.05$).

A comparison was also made of the potentiating ability of cocaine and guanethidine. At equimolar concentrations (3×10^{-5}M) there was no significant difference between the degree of potentiation by either drug on both the longitudinal and circular muscle.

α-Adrenoceptor blockade

Phenoxybenzamine (0·5–1 μg ml^{-1}) and phentolamine (1–5 μg ml^{-1}) abolished completely the response of the vein to added noradrenaline.

Acetylcholine

The mean cumulative dose response curves for pressure and tension in thirteen veins to acetylcholine are presented graphically in Fig. 4. In contrast to the findings of McConnell & Roddie (1970) with bovine mesenteric vein in which the circular muscle does not respond to acetylcholine (2 μg ml^{-1}) the circular muscle of dog mesenteric vein does respond to smaller doses. The minimum effective dose is about 0·1 μg ml^{-1}. As in the case of noradrenaline, the pressure curve does not rise with the tension curve indicating that the circular muscle is not as sensitive as the longitudinal muscle to acetylcholine. Atropine 1 μg ml^{-1} abolished the responses to acetylcholine.

Histamine

Noradrenaline is a much more powerful stimulant of the anterior mesenteric vein than histamine (Table 1). In six experiments the response to histamine, especially

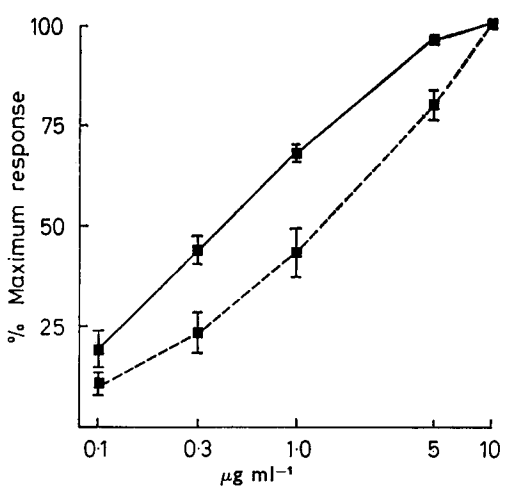

FIG. 4. The mean cumulative dose response curves for pressure (■ – – ■) and tension (■—■) in 13 veins to acetylcholine. Vertical bars show s.e. The ordinate shows the observed responses as a percentage of the maximum response.

of the circular muscle, was very weak. Progressive tachyphylaxis of the response of the longitudinal muscle on repeated exposure to histamine occurred in all venous segments examined. A typical experiment of four successive cumulative dose response curves to histamine is shown in Fig. 5. Following each curve the bath fluid was changed three times and 30 min was allowed to elapse. There was little change in the sensitivity of the vein segment to noradrenaline at the end of the experiment as shown in Fig. 5. Because of the feeble response of the circular muscle to histamine, the possibility of its showing tachyphylaxis to histamine was not explored.

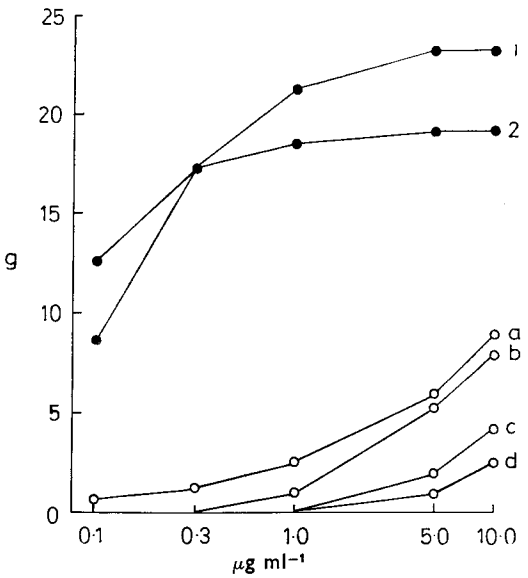

FIG. 5. Four successive cumulative dose response curves of the longitudinal muscle to histamine which show progressive tachyphylaxis (a, b, c and d). Following each curve the perfusing fluid was changed three times and 30 min was allowed to elapse. (1) shows the cumulative dose response curve to noradrenaline at the start of the experiment and (2) the curve after the histamine trials.

Angiotensin

Tachyphylaxis of the response of the longitudinal muscle to angiotensin is shown in Fig. 6A. As shown in this experiment the longitudinal muscle responded to the first dose of angiotensin (0·1 μg ml^{-1}) but did not respond to further doses of 0·3 and 1 μg ml^{-1}. After changing the fluid in the system three times, and waiting 30 min angiotensin was still without effect on the longitudinal muscle. In two other veins an identical behaviour pattern to angiotensin was found. The circular muscle did not respond to angiotensin even in doses as high as 1 μg ml^{-1} in the three experiments.

5-HT

Both the longitudinal and circular muscle of the vein segment responded to 5-HT (Table 1). In the initial cumulative dose-response curve of the experiment shown in Fig. 6B the maximum tension achieved was about 14·7 g and the perfusion pressure increased by about 3 mm Hg. After the fluid in the system had been changed three times and 30 min had elapsed, the second cumulative dose response curve showed a maximum tension rise of only 6·6 g, about 40% of the initial response and virtually

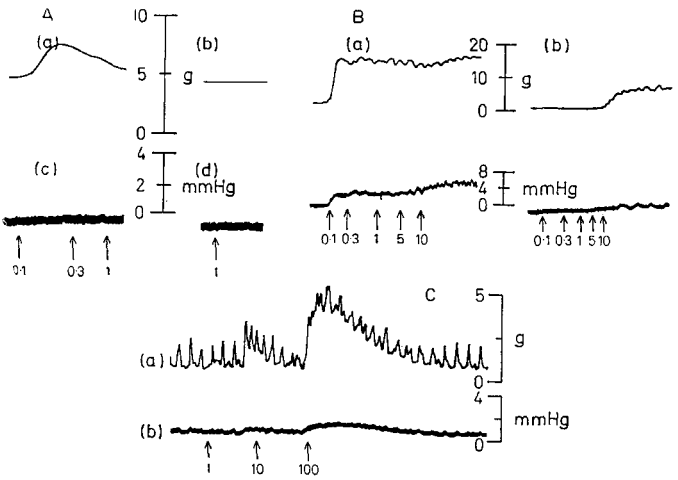

FIG. 6A. (a) an initial response of the longitudinal muscle to angiotensin, 0·1 μg ml^{-1} but no further response to higher doses. After changing the perfusing fluid three times and waiting 30 min the longitudinal muscle was still unresponsive to angiotensin (b). In (c) and (d) the circular muscle was unresponsive to all doses of angiotensin tested. Doses in μg ml^{-1}.

FIG. 6B. (a) the initial response of the longitudinal and circular muscle to 5-HT and (b) the response obtained after three washouts and an interval of 30 min, which show partial tachyphylaxis.

FIG. 6C. (a) shows the transient responses of the longitudinal muscle to large doses of vasopressin and (b) shows a feeble response of the circular muscle to vasopressin, 100 mu ml^{-1}. Doses in mu ml^{-1}.

no change in perfusion pressure. Partial tachyphylaxis to 5-HT occurred in the four experiments in which it was tested. The feeble response, especially of the circular muscle, contrasts with the findings of McConnell & Roddie (1970) for bovine mesenteric veins.

Atropine sulphate (10 μg ml^{-1}) did not block the action of 5-HT on the vein. In the presence of 2-bromo-D-lysergic acid diethylamide (0·1 μg ml^{-1}) the responses of the longitudinal and circular muscle to 5-HT were abolished. This suggests the presence of only D-receptors for 5-HT in the anterior mesenteric vein.

Vasopressin

Large doses of antidiuretic hormone have a vasopressor action. This action seems to have two components, a direct one on vascular smooth muscle and an indirect one through the potentiation of the vasopressor action of catecholamines (Bartelstone & Nasmyth, 1965). Vasopressin (0·5 mu ml^{-1}) had no potentiating effect on the response of the vein to added noradrenaline. In very large doses (10–100 mu ml^{-1}) the longitudinal muscle of the vein contracted as shown in Fig. 6C. The response of the circular muscle was feeble.

Isoprenaline

Isoprenaline partially reduced the pressure and tension induced by acetylcholine in the vein segment. The experimental procedure was as follows: a vein segment pretreated with phentolamine, 5 μg ml^{-1}, to block the α-adrenoceptors, was exposed to acetylcholine 5 μg ml^{-1}. When the response had developed (3 min) isoprenaline was added and both tension and pressure recorded for a further 10 min. In the

experiment shown in Fig. 7 the pressure had fallen to 44% and the tension to 81% of the initial response to acetylcholine in the 10 min. In the control period of observation as shown in Fig. 7, in the absence of isoprenaline the response to acetylcholine was sustained over the 10 min period. The prior application of the β-blocking agent propranolol (1–5 μg ml^{-1}) abolished this relaxing effect of isoprenaline. This suggests the existence of β-adrenoceptors in both the longitudinal and circular muscle.

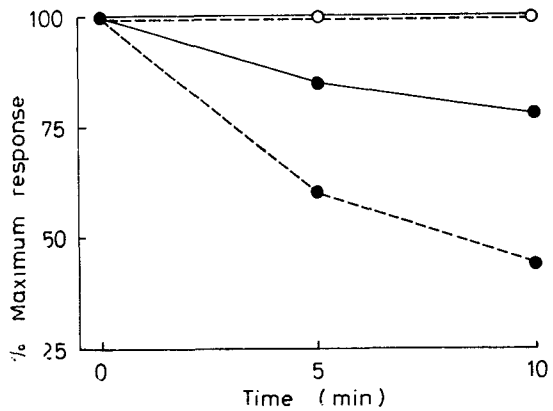

FIG. 7. In a vein pretreated with phentolamine (5 μg ml^{-1}) and exposed to acetylcholine (5 μg ml^{-1}) maximum tension (●——●) and pressure (●– – –●) developed and was sustained for at least 10 minutes. In the presence of isoprenaline, 5 μg ml^{-1}, the tension fell to 81% and the pressure to 44% of maximum in 10 min.

DISCUSSION

The potentiation of responses to catecholamines by cocaine is generally thought to be due to inhibition of the amine uptake mechanism in noradrenergic nerves (Furchgott, Kirpekar & others, 1963; Muscholl, 1966; Iversen, 1967). The potentiation by cocaine of both the longitudinal and circular muscle responses to noradrenaline in our experiments suggests the presence of noradrenergic nerves in both these muscle layers, This was confirmed by fluorescent microscopy.

Guanethidine, like cocaine, potentiated the responses of both muscle layers to noradrenaline. There was no significant difference between the degree of potentiation observed with cocaine and guanethidine. Many theories could be offered to explain why cocaine and guanethidine potentiate the responses of the circular muscle to a lesser extent than those of the longitudinal fibres. Bearing in mind the probable site of action of these two agents, it is possible that the circular muscle is not as richly innervated as the longitudinal with noradrenergic nerves.

Holman & McLean (1967) found that contractions of the longitudinal muscle in sheep mesenteric vein to acetylcholine were abolished by phentolamine and atropine. We found phentolamine did not block the response to acetylcholine which was, however, completely abolished by atropine. These findings appear to rule out the suggestion that acetylcholine might act in this situation by releasing noradrenaline from noradrenergic C fibres (Ferry, 1963).

Many workers, including Hughes & Vane (1967) and Sutter (1965) have reported rapid development of tachyphylaxis in venous muscle to angiotensin. In our experi-

ments with the dog anterior mesenteric vein, the longitudinal muscle after an initial response failed completely to respond further (Fig. 6A). This accords with the findings of Somlyo & Somlyo (1966) who reported tachyphylaxis to angiotensin in strips of canine mesenteric veins.

While tachyphylaxis of vascular muscle to angiotensin appears to be well established, tachyphylaxis to 5-HT has not been commonly reported. Many studies of venous muscle including those of Sutter (1965) and Hughes & Vane (1967) do not report any tachyphylaxis of venous muscle to 5-HT. However, Maengwyn-Davies, Crisp & others (1969) observed the progressive development of tachyphylaxis to 5-HT in guinea-pig aortic strips. We observed partial tachyphylaxis to 5-HT (Fig. 6B).

Although longitudinal muscle of the dog anterior mesenteric vein contracted to histamine, the circular muscle response was feeble. As found by Hughes & Vane (1967), who used rabbit portal vein, the response of the longitudinal muscle to histamine was much weaker than to noradrenaline (Table 1). We found, as with 5-HT, progressive tachyphylaxis of the longitudinal muscle to histamine. The significance of the development of tachyphylaxis to the above substances in the normal function of the anterior mesenteric vein of the dog remains to be determined.

Webb-Peploe & Shepherd (1969) have shown that venodilation in the superficial limb veins of the dog consequent on β-adrenoceptor stimulation is proportional to the initial degree of venous tone. Crotty, Hall & Sheehan (1969) have also shown that isoprenaline applied to the unstimulated saphenous vein of the dog, had no demonstrable effect, but if the vein were constricted by noradrenaline, isoprenaline caused prompt venodilation. Similarly, in our experiments the existence of β-adrenoceptors in dog anterior mesenteric vein could only be demonstrated in a stimulated vein.

Acknowledgement

This paper is based on work submitted by P. C. O'C. to the National University of Ireland for the degree of M.Sc. and the authors wish to thank the Irish Heart Foundation for a grant to W.J.H. towards equipment.

REFERENCES

BARTELSTONE, H. J. & NASMYTH, P. A. (1965). *Am. J. Physiol.*, **208**, 754–762.

BLOOM, W. & FAWCETT, D. W. (1968). *A Textbook of Histology*, 9th edn. Chapter 13, "Blood Vascular System", pp. 258–389. Philadelphia: W. B. Saunders.

BOURA, A. L. & GREEN, A. F. (1962). *Br. J. Pharmac. Chemother.*, **19**, 13–41.

CROTTY, T. P., HALL, W. J. & SHEEHAN, J. D. (1969). *J. Physiol.*, **205**, 6P.

DAY, M. D. & RAND, M. J. (1961). *Br. J. Pharmac. Chemother.*, **17**, 245–260.

DE LA LANDE, I. S. & RAND, M. J. (1965). *Aust. J. exp. Biol. med. Sci.*, **43**, 639–656.

FERRY, C. B. (1963). *J. Physiol.*, **167**, 483–504.

FOSTER, R. W. (1967). *Br. J. Pharmac. Chemother.*, **31**, 466–482.

FURCHGOTT, R. F. (1955). *Pharmac. Rev.*, **7**, 183–265.

FURCHGOTT, R. F., KIRPEKAR, S. M., RIEKAR, M. & SCHWAB, A. (1963). *J. Pharmac. exp. Ther.*, **142**, 39–58.

GOKHALE, S. D., GULATI, O. D. & KELKAR, V. V. (1967). *Br. J. Pharmac. Chemother.*, **30**, 445–462.

HOLMAN, M. E. & MCLEAN, A. (1967). *J. Physiol.*, **190**, 55–69.

HUGHES, J. & VANE, J. R. (1967). *Br. J. Pharmac. Chemother.*, **30**, 46–66.

IVERSEN, L. L. (1967). *The uptake and storage of noradrenaline in sympathetic nerves.* Cambridge: Cambridge University Press.

LI, P. C. (1940). *J. Path. Bact.*, **50**, 121–136.
MACMILLAN, W. H. (1959). *Br. J. Pharmac. Chemother.*, **14**, 385–391.
MAENGWYN-DAVIES, G. D., CRISP, T. M., DANIEL, E. E. & THOA, N. B. (1969). *Archs int. Pharmacodyn. Thér.*, **178**, 344–350.
MCCONNELL, J. G. & RODDIE, I. C. (1970). *J. Physiol.*, **207**, 82P–83P.
MCCUBBIN, J. W., KANEKO, Y. & PAGE, I. H. (1961). *J. Pharmac. exp. Ther.*, **131**, 346–354.
MCGREGOR, D. D. (1965). *J. Physiol.*, **177**, 21–30.
MUSCHOLL, E. B. (1966). *Pharmac. Rev.*, **18**, 551–559.
ROGERS, L. A., ATKINSON, R. A. & LONG, J. P. (1966). *J. Pharmac. exp. Ther.*, **151**, 313–320.
SOMLYO, A. V. & SOMLYO, A. P. (1966). *Am. Heart. J.*, **71**, 568–570.
SUTTER, M. C. (1965). *Br. J. Pharmac. Chemother.*, **24**, 742–751.
WEBB-PEPLOE, M. M. & SHEPHERD, J. T. (1969). *J. clin. Invest.*, **48**, 1328–1335.
WILLIAMSON, I. M. (1969). *J. Physiol.*, **202**, 112P–113P.