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Actions of neurotransmitters and peptides on longitudinal and circular muscle of the rat portal vein

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The reactivity of longitudinal and circular muscle of the rat portal vein to noradrenaline, acetylcholine, 5-hydroxytryptamine, substance P, angiotensin II and neurotensin was compared. Longitudinal muscle was prepared as longitudinal strips and circular muscle was studied as transversally cut rings. Longitudinal muscle was more sensitive than circular muscle to acetylcholine, 5-hydroxytryptamine and angiotensin II, whereas both muscle layers were equally sensitive to noradrenaline and substance P. Circular muscle was generally unresponsive to neurotensin. Differential sensitivity of longitudinal and circular muscle layers suggests that these two muscle layers do not necessarily operate in unison.

When compared with other veins of comparable size the portal vein is atypical in being composed to two mutually perpendicular muscle layers: the longitudinal and the circular (Ts'ao et al 1970). Despite this disposition of smooth muscle, the actions of vasoactive agents have been characterized mainly on longitudinal muscle (Blair-West et al 1971; Carruba et al 1973; Weston & Golenhofen 1976; Rioux et al 1980), although several studies have demonstrated differences in the responsiveness of circular and longitudinal muscle to a variety of agents on portal and/or mesenteric veins isolated from cow (McConnell & Roddie 1970), rabbit (Sutter 1965) and dog (Hall & O'Connor 1973). Even though the portal vein of the rat is widely used to characterize the actions of vasoactive agents on vascular smooth muscle, a comparative study of the longitudinal and circular muscle of this species has not been undertaken. Furthermore, an understanding of the role of these two muscle layers can be attained only if their responses to vasoactive agents are characterized.

The present investigation examines the concentration-effect relationships of various neurotransmitters, hormones and neuropeptides on circular and longitudinal muscle of the rat portal vein. Both muscle layers responded to all ligands, although circular muscle is generally less responsive to some of these agents.

METHODS

Wistar rats (200–250 g) of either sex were killed by a blow on the neck and the portal vein with a short segment of the anterior mesenteric vein removed. A vein was cut either into three longitudinal strips (3–4 mm in width), or transversally into five rings (2–3 mm in width) which were tied together in series to form a chain. Both types of preparations were 1–1·5 mm in length when suspended under 0·5 g of isometric tension. Increases in tension exerted by strip and ring preparations were taken to represent contractions of longitudinal and circular muscle, respectively. All tissues were mounted in a 10 ml tissue bath containing a modified Krebs solution (mM): NaCl 118·7, KCl 4·7, $CaCl_2$ 2·8, KH_2PO_4 1·2, $NaHCO_3$ 15·5, $MgCl_2$ 1·19, glucose 10·1. The physiological solution was bubbled with 95% O_2–5% CO_2 (pH 7·4–7·5) and maintained at 33 °C. All compounds tested, diluted with Krebs buffer, were added in a small volume (less than 10 µl) and the reaction was allowed to proceed for 3 min. Following compound washout at least 10 min incubation was allowed before another addition.

The responses of the veins were quantified by integrating electronically the force generated in minute periods. The force generated, as a result of spontaneous contractile activity of longitudinal muscle, and sometimes circular muscle, tended to give an overestimation of the actual force generated by the vein consequent to the action of a vasoactive agent. To determine the actual contribution of an agonist to the force generated, the mean spontaneous force min^{-1}, determined for a 5 min period before ligand application, was subtracted from the mean total force generated during the second and third minute of ligand application to yield the net force per minute.

Complete dose-response curves for six compounds—noradrenaline (NA), acetylcholine (ACh), 5-hydroxytryptamine (5-HT), angiotensin II (AII), substance P (SP) and neurotensin—were contructed with both strip and ring preparations. On the basis of the maximal response for a ligand on any one preparation, saturation curves were constructed.

The relative activity (R.A.) of the various ligands was determined in relation to the ligand inducing the greatest mean maximal force.

Noradrenaline and acetylcholine were purchased from Sigma; 5-hydroxytryptamine from Fluka; angiotensin II from Beckman; substance P and neurotensin from Vega Biochemicals.

RESULTS

Rhythmical spontaneous contractions, with a frequency of 3–5 contractions min^{-1} were always observed with longitudinal muscle (Fig. 1A–1C). With circular muscle spontaneous contractions of variable frequency (Fig. 1D) were noted when a tissue was initially suspended in the tissue bath, but these contractions tended to decrease (Fig. 1E) with time or after the addition of a high concentration of a vasoactive agent. This initial spontaneous activity was generally an indication that a ring preparation would respond to most compounds tested; in their absence at the beginning of the experiment poor responses to vasoactive agents were observed and such preparations were discarded. Nevertheless, even if spontaneous contractions of circular muscle gradually diminished, the responsiveness to the agonists was not decreased significantly. In contrast, a decrease in spontaneous phase activity of longitudinal muscle appeared to result in a marked attenuation of the responses of this muscle layer to the agonists (results not shown).

All compounds tested caused an initial increase in contraction frequency of longitudinal muscle (Fig. 1A–1C) such that individual contractions did not return to the base line. This type of behaviour was suggestive of a tonic component in the contracture, although the true tonic component of vasoconstrictor action could not be discerned. Some differences in the actions of the various agonists on longitudinal muscle may be noted. Firstly, ACh (Fig. 1C) and NA have a more rapid onset than 5-HT or the peptides. Secondly, neurotensin (Fig. 1A) had a slow onset and a rapid fade which was undoubtedly a reflection of the marked tachyphalaxis exhibited by longitudinal muscle to this peptide. The tachyphalaxis to neurotensin could be avoided by allowing at least 30 min between consecutive applications of the peptide. Occasionally, a longitudinal muscle preparation was encountered that was totally unresponsive to neurotensin. Lastly, whereas a rapid return to normal spontaneous activity was observed upon washout of ACh (Fig. 1C) and NA, large phasic contractions of low frequency persisted for 4 to 5 min upon washout of AII (Fig. 1B), 5-HT and SP.

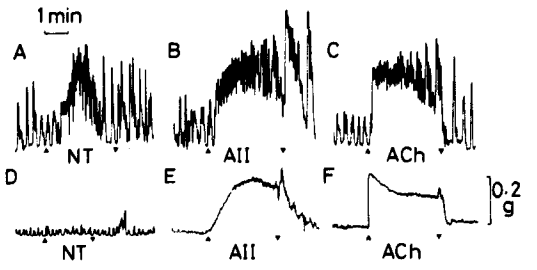

FIG. 1. Responses of neurotensin, angiotensin and acetylcholine on longitudinal (A–C) and circular (D–F) muscle of the rat portal vein. Drugs, at concentrations equal to their ED50, were added at upward arrow and washed out at downward arrow. All responses are shown at the same force (0·2 g) and time (1 min) scales.

Agonist action on circular muscle, even in the presence of phasic contractions, was characterized by a tonic-like contracture (Fig. 1E and 1F). With the exception of ACh (Fig. 1F), the rate of rise to a maximal effect appeared to be much slower for circular muscle than for longitudinal muscle. Neurotensin was generally inactive on circular muscle (Fig. 1D), but in 2 out of 10 preparations NT did induce a contraction of this muscle layer. In contrast to longitudinal muscle, the circular muscle quickly attained base line upon ligand washout; a notable exception, however, is the washout response to AII (Fig. 1E).

Saturation curves for the actions of the six agonists

Table 1. ED50 values and relative activities (R.A.) of vasoactive ligands on longitudinal (strips) and circular (rings) muscle preparations of the rat portal vein.

Agonist – Preparation	ED50* (pD_2)	R.A.
AngiotensinII		
– strips	8·44 ± 0·04	1·00
– rings	7·96 ± 0·14	0·70
Neurotensin		
– strips	7·60 ± 0·11	0·35
– rings	(—)	(—)
Noradrenaline		
– strips	6·61 ± 0·11	0·87
– rings	6·48 ± 0·09	1·00
Substance P		
– strips	6·04 ± 0·05	0·73
– rings	5·97 ± 0·06	0·70
5-Hydroxytryptamine		
– strips	5·34 ± 0·11	0·35
– rings	4·89 ± 0·06	0·27
Acetylcholine		
– strips	6·07 ± 0·12	0·68
– rings	4·84 ± 0·08	0·51

* ED50 = pD_2 in molar concentrations.

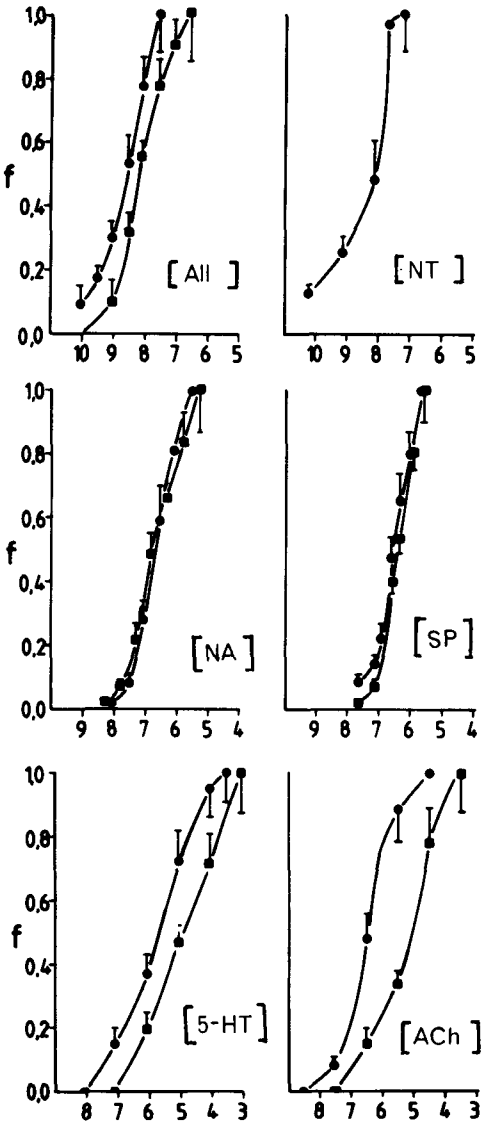

FIG. 2. Saturation curves for angiotensin II (AII), neurotensin (NT), noradrenaline (NA), substance P (SP), acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) on longitudinal (●——●) and circular (■——■) muscle of the rat portal vein. Abscissa, negative logarithm of agonist; ordinate, effect expressed as net force (f). ($n \geq 5$; $x \pm$ s.e.m.).

on longitudinal and circular muscle are shown in Fig. 2. The calculated ED50 values and relative activities are summarized in Table 1.

The actions of NA, as well as those of SP, on longitudinal and circular muscle, were identical with regard to superposition of the saturation curves. Angiotensin II, ACh and 5-HT were less potent on circular muscle than on longitudinal muscle, and owing to the general absence of a neurotensin effect on circular muscle, a saturation curve for this peptide is shown only for longitudinal muscle.

Angiotensin II was the most potent agonist on longitudinal muscle, and NA the most potent on circular muscle. Compounds which demonstrated lower ED50 values on circular muscle than on longitudinal muscle (ACh and AII) also exhibited a lower relative activity on this muscle layer. Substance P, however, had similar relative activities on the two muscle layers.

The maximal forces generated by the two preparations were comparable; AII, the most potent agonist on strips, produced $0{\cdot}37 \pm 0{\cdot}05$ g min^{-1} and NA, the most potent on rings, yielded $0{\cdot}37 \pm 0{\cdot}04$ g min^{-1}. The strip preparation, however, represents only a third of the total longitudinal muscle layer, and thus this muscle layer, if intact, has the capacity to generate approximately three times as much force as the circular muscle. A whole vein preparation of the longitudinal muscle generated $0{\cdot}95 \pm 0{\cdot}06$ g min^{-1} in the presence of a maximal concentration of noradrenaline.

DISCUSSION

Longitudinal muscle of portal and mesenteric veins in all species studied to date, with the possible exception of the sheep (Blair-West et al 1971), exhibit spontaneous rhythmical contractions; circular muscle on the other hand, is generally quiescent (McConnell & Roddie 1970) or exhibits a minimal amount of rhythmic activity (Sutter 1965). This latter type of activity was observed with circular muscle from rat portal vein. The absence of a minimal amount of rhythmicity in circular muscle may be indicative of a disturbance of intermuscle relationships during tissue preparation (Hall & O'Connor 1973), or possible damage to the endothelial cell layer during ring preparation (Furchgott et al 1981). Nevertheless, it is not known whether circular muscle possesses an intrinsic activity in-vivo, and thus the possibility should be considered that myogenic activity in these two muscle layers is controlled by different mechanisms.

The gradual disappearance of the spontaneous contractions of circular muscle during the course of the experiment, despite the retention of pharmacological responsiveness, suggests that circular muscle was not tightly coupled to a pacemaker region nor was pacemaker activity an absolute requirement for the induction of a mechanical response. In contrast, the responsiveness of longitudinal muscle to vasoconstrictors was more intimately related to the

presence of phasic contractions. This relationship is probably due to spontaneous contractions being coupled to functional voltage-dependent calcium channels (Johansson & Somlyo 1980); the availability of these channels would thus determine, at least to a certain extent, the response of the longitudinal muscle to an agonist. An additional observation which suggests that a different excitation-contraction coupling mechanism operates on circular muscle is the essentially tonic-like contracture produced by compounds on this muscle layer. Tetanic fusion in longitudinal muscle only is observed with high doses of agonists. Golenhofen (1981) has defined two distinct systems for the activation of smooth muscle: the phasic or P-system and the tonic or T-system. The longitudinal muscle of the portal vein belongs to the P-system since the contractions induced by agonists are tightly coupled to changes in membrane potential and these actions are blocked by the calcium antagonist nifedipine (Golenhofen 1981). A small proportion of agonist action on longitudinal muscle may be due to the activation of the T-system. Based exclusively on the type of responses obtained to contracting agonists, it is suggested that smooth muscle cells of the circular muscle layer possess the T-system of activation. These muscle cells would be expected to possess the following characteristics: resistance of the contracting effects of angonists to nifedipine and a slight degree of electrical coupling. Thus, according to the schemata for activation of smooth muscle cells proposed by Bolton (1979) the circular muscle smooth muscle cells may be regulated by receptor-operated channels rather than voltage-dependent channels.

With the exception of neurotensin, all agonists tested consistently produced contraction of both longitudinal and circular muscle. The general absence of a response of circular muscle to neurotensin may indicate a marked desensitization of this muscle layer by blood-born peptide (Carraway & Leeman 1976). A marked tachyphalaxis of longitudinal muscle to this peptide has been noted previously (Helle et al 1980). The absence of responses to contracting agonists of both the circular or longitudinal muscle has been noted in several species. Circular muscle of the dog portal vein is unresponsive to AII (Hall & O'Connor 1973) and the cow mesenteric vein does not react to ACh (McConnell & Roddie 1970). Longitudinal muscle of rabbit (Carruba et al 1973) and of cow (McConnell & Roddie 1970) are not responsive to 5-HT.

The enhanced sensitivity and responsiveness of the longitudinal muscle layer to ACh and 5-HT may be a reflection of the proximity of receptors for these agonists to sites for their release within the vascular wall. ACh-containing nerve fibres are found exclusively at the junction of the adventitia and the media (De Luca et al 1982). Mastocytes containing 5-HT are localized exclusively to the adventitial layer (Booz 1971). The presence of nerve fibres containing NA (Johansson et al 1970) and SP (Barja & Mathison 1982) within the neve plexus separating the two muscle layers, suggests that release of either the amine or the neuropeptide from intramural nerves would activate both muscle layers.

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