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SPONTANEOUS ACTIVITY IN ISOLATED BOVINE MESENTERIC LYMPHATICS

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SUMMARY

1. Longitudinal tension was measured in isolated segments (approximately 20 mm in length and 2 mm in diameter) of bovine mesenteric lymphatic vessels.
2. Seventeen out of thirty-nine vessels showed regular spontaneous rhythmic contractions, the most common frequency being about 2/min.
3. The strength of each contraction was related to the duration of the preceding pause, a longer pause being associated with a stronger contraction.
4. Noradrenaline increased the frequency and decreased the amplitude of the contractions. This effect was abolished by α-receptor blockade with phentolamine.
5. Isoprenaline slowed the frequency of the contractions. This effect was abolished by β-receptor blockade with propranolol.
6. Acetylcholine had little effect on the rate of spontaneous contractions.

INTRODUCTION

In 1927, Florey noted that rhythmical contractions occurred in the lacteals of the rat and that the rate of these contractions was increased by local application of adrenaline and by stimulation of the splanchnic nerves. Although this was not found in most of the other species studied, Smith, in 1949, demonstrated that the popliteal lymphatics in rats, mice and guinea-pigs behaved similarly to locally applied adrenaline.

In 1969, Williamson found that noradrenaline had very little effect on the smooth muscle of perfused bovine mesenteric lymphatics although other vasoactive drugs, such as 5-hydroxytryptamine and bradykinin, produced strong contractions. This was surprising, since she showed that the walls of these vessels contained catecholamines using the Falck technique which demonstrates catecholamine-formaldehyde complexes by fluorescence. However, most of the preparations perfused by Williamson did not show spontaneous activity and when this is present a different picture is obtained. The present experiments were designed to study spontaneous activity in these vessels and how such activity is modified by catecholamines.

METHODS

Lymphatic vessels can be seen as small opalescent tubes running on the surface of bovine mesentery immediately under the peritoneum. They usually travel separately from the mesenteric arteries and veins. Segments of these lymphatics, measuring 2–3 mm in diameter and 2–3 cm long, were dissected from the fresh mesenteries of recently slaughtered cattle and mounted in a water-jacketed organ bath. A thread attached the upper end of the preparation to the arm of an isometric mechanotransducer (Statham model UC3) which responded to changes in the longitudinal tension of the vessel. The output of the mechanotransducer was amplified and recorded on a pen recorder (Devices model M2).

The bath was perfused with a Krebs bicarbonate solution with the following composition (g/l.): NaCl 6·9; $NaHCO_3$ 2·1; KCl 0·35; NaH_2PO_4 0·16; $CaCl_2$ 0·28; $MgCl_2$ 0·1; glucose 1·0. The perfusate was preheated to 37° C and aerated with a 95 % O_2–5 % CO_2 mixture.

After the lymphatics were installed in the organ bath, the tension was adjusted to 0·1 g. The preparation was then allowed a period of one to 2 hr to equilibrate. If spontaneous activity developed during this time, and had become regular in rate and amplitude, the effects of drugs were studied. The drugs used were: noradrenaline bitartrate (NOR), isoprenaline sulphate (IPN), acetylcholine hydrochloride (ACh), phentolamine mesylate and propranolol hydrochloride. Concentrations of noradrenaline refer to the base, those of the others to their respective salts.

RESULTS

Occurrence of spontaneous activity

Thirty-nine vessels were used and thirty of these showed spontaneous contractions. Fig. 1 shows recordings of three varieties of activity which were encountered. The most common variety was that seen in *a*. Here the activity was regular in rate and amplitude. It consisted of fast contractions followed by slightly slower relaxations and a comparatively long pause before the next contraction. The frequency of the contractions was about 2/min. Less commonly, irregular contractions of the types seen at *b* and *c* were recorded.

Fig. 2 shows a histogram representing the resting rates of contraction in fifteen of the vessels displaying regular activity. These values are averages for a period of at least 5 min duration before any stimulus was applied. It can be seen that the values for frequency are distributed about a mean of 2·0–2·5 contractions/min and that the values range from 1·0 to 4·0/min.

Fig. 3 shows a trace suggesting that the strength of individual contractions was related to their frequency. The upper half of the diagram is a graph of the instantaneous rate, calculated for each contraction from the interval between it and the previous contraction. Two irregularities in frequency occurred. On the left the frequency fell. The longer pause before the late contraction was associated with an increase in its strength. When

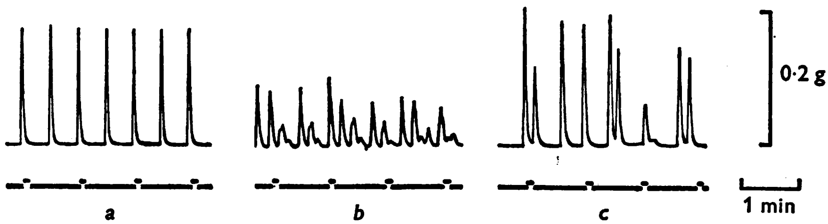

Fig. 1. Spontaneous activity in bovine mesenteric lymphatics. Most commonly, activity was regular in rate and amplitude (*a*) but irregular activity (*b* and *c*) was seen in some preparations.

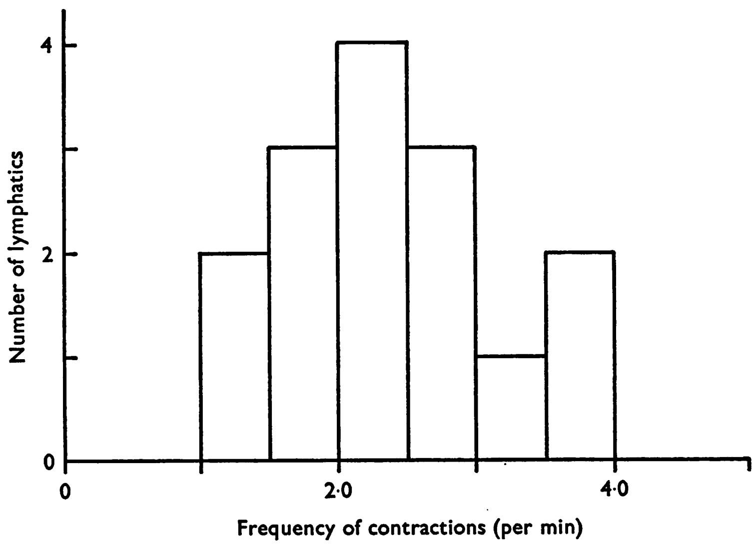

Fig. 2. The frequency of spontaneous contractions in bovine mesenteric lymphatics. The most common frequency was about 2/min.

the frequency rose, the shorter pause before the early contraction was associated with a reduction in its amplitude.

Fig. 4 is a scatter diagram showing the relationship between the two values for each contraction in the complete experiment. The correlation coefficient, r, equals 0·79 and the probability that this value arose by chance, the true value being zero, is less than 1 in 1000. The regression equation for predicting amplitude (y) from an observed value of interval (x) was $y = 0{\cdot}005257x - 0{\cdot}023$. Seven lymphatics showed irregularities such as this and in all cases there was a significant correlation between amplitude and interval.

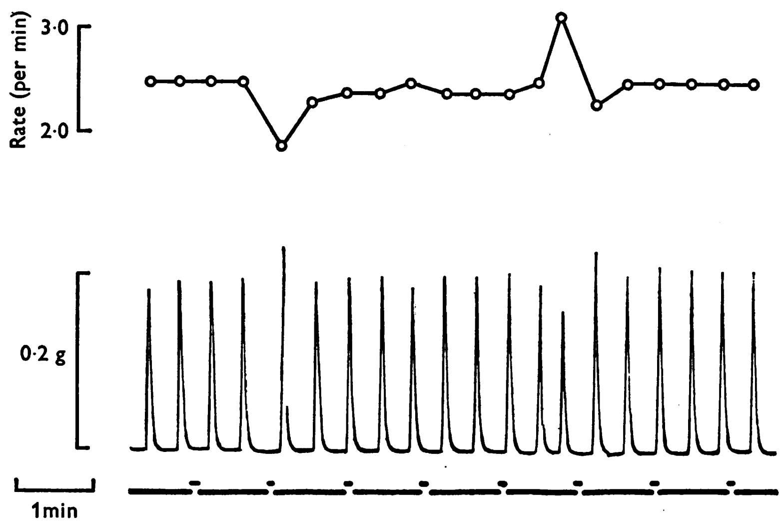

Fig. 3. The effect of spontaneous changes in rate on the strength of contraction of a bovine lymphatic. A decrease in the rate of contraction was associated with an increase in amplitude and vice versa.

Effects of catecholamines

Fig. 5 shows the effect of noradrenaline on spontaneous activity. At the beginning of the run, the lymphatic contracted rhythmically at a rate of about 2 beats/min. A 5 ng/ml. solution was perfused through the bath during the period indicated by the arrows. Its effect was to about double the rate of contractions. The amplitude of contractions was reduced. When the noradrenaline was washed out, the frequency fell to a little below the resting rate before returning to normal.

Fig. 6 shows that this effect is dose dependent. Three doses of noradrenaline were given: 1, 5 and 25 ng/ml. The effect of the 1 ng/ml. solution was to slightly increase the rate of contraction and slightly reduce the amplitude. These effects were more marked by the 5 ng/ml. dose. The 25 ng/ml. dose caused the frequency of the contractions to increase to about 10/min. However, at this high frequency, each contraction has a very small amplitude and can just be seen as a ripple on the trace.

This effect of noradrenaline could be abolished by the α-receptor blocking agent phentolamine in the perfusate, at a concentration of 1 μg/ml. (Table 1).

Ten vessels were used and nine of these reacted as described above, although in several cases the slowing after the drug was washed out of the bath was more marked. One vessel reacted to noradrenaline by decreasing its rate of beating.

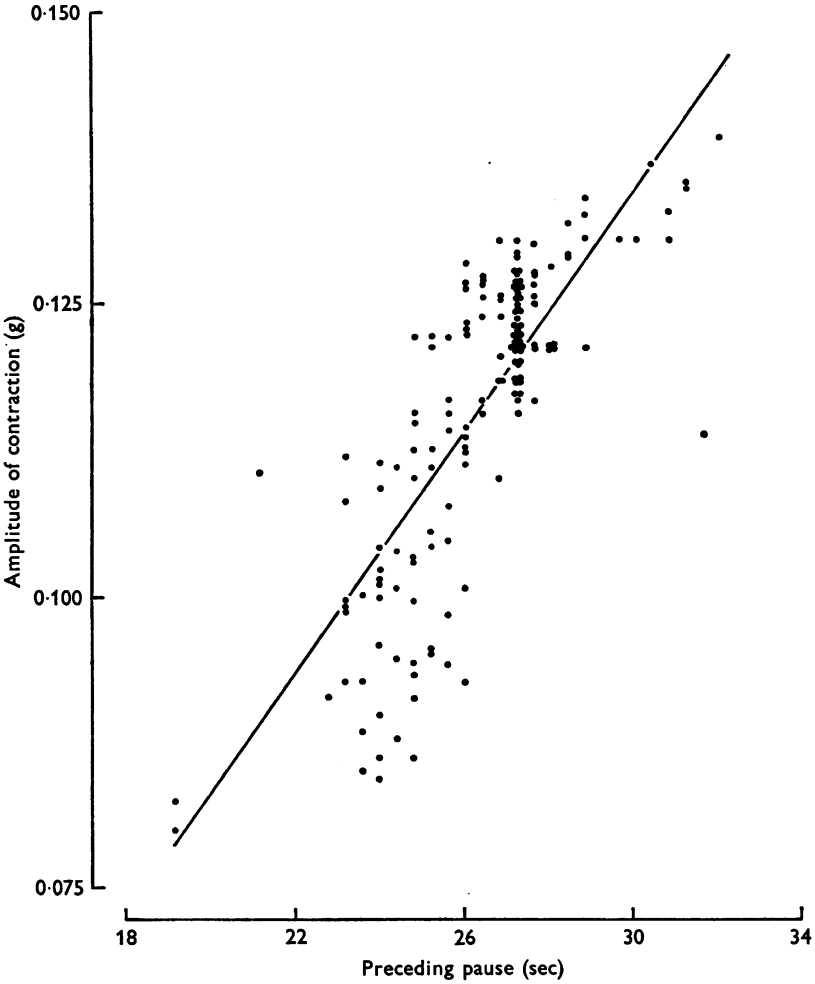

Fig. 4. The relationship between the amplitude of contraction and the duration of the preceding pause in a spontaneously beating bovine lymphatic. There was a significant correlation between amplitude and the preceding pause (r = 0·79).

Fig. 7 shows the reaction to isoprenaline. On the left, a 1 ng/ml. solution caused the frequency to decrease from about 2 to about 1 beat/min. On the right, a 5 ng/ml. solution caused a long pause, with an even larger

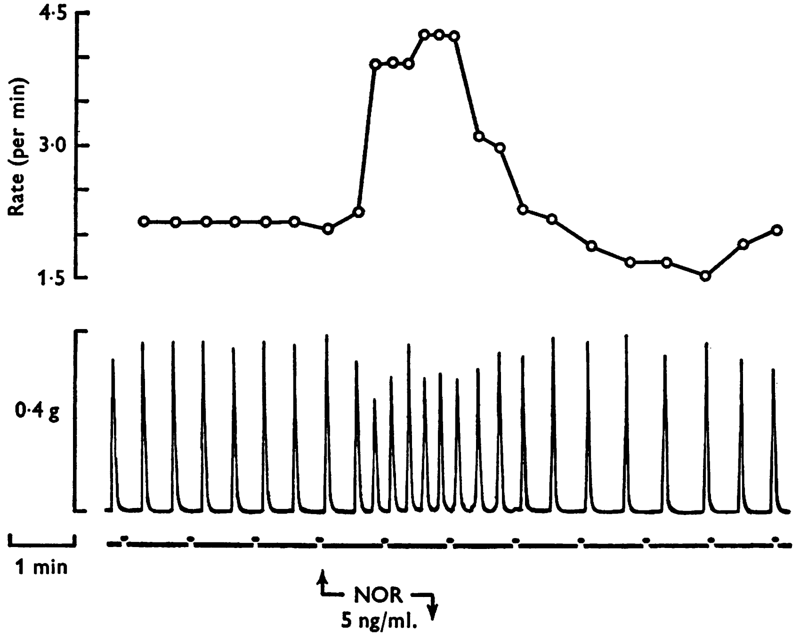

Fig. 5. The effect of noradrenaline (5 ng/ml.) on the rate and strength of contraction of a bovine lymphatic. When noradrenaline (NOR) was added to the bath there was an increase in rate associated with a slight decrease in the amplitude of the contractions.

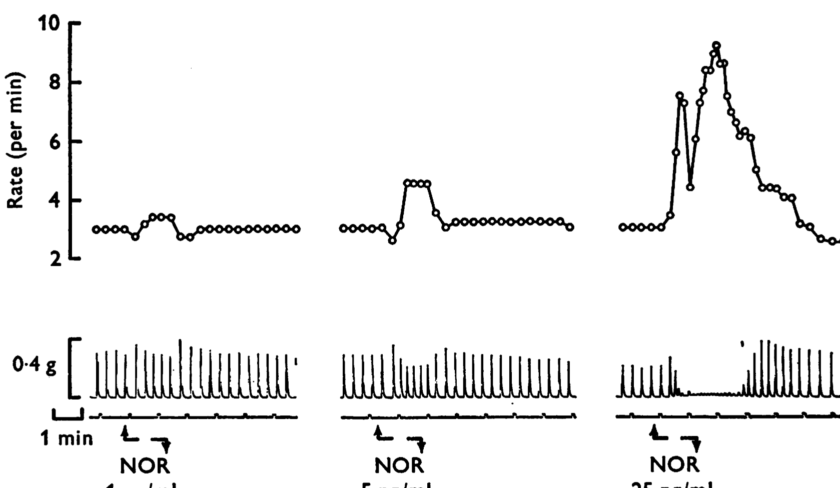

Fig. 6. The effect of increasing doses of noradrenaline on the rate and strength of contractions of bovine lymphatics. The increase in frequency with noradrenaline (NOR) was dose dependent.

decrease in rate. After the drug was washed out, the amplitude of the contractions was transiently decreased. Further increases in dose abolished spontaneous contractions for longer periods.

This effect of isoprenaline could be blocked by the β-receptor blocking agent, propranolol, in the perfusate at a concentration of 1 μg/ml. (Table 1). Seven vessels were tried and all reacted in this way.

TABLE 1. The effect of phentolamine (1 μg/ml.) and propanolol (1 μg/ml.) on the increase and decrease in rate of contraction caused by noradrenaline and isoprenaline respectively. The doses of noradrenaline before and after phentolamine were 25 and 100 μg/ml. respectively. The doses of isoprenaline before and after propanolol were 5 and 20 μg/ml. respectively

Change in rate of contraction with noradrenaline (%)

	Before phentolamine	After phentolamine
Expt. 1	+107	+8
Expt. 2	+95	+4
Average	+101	+6

Change in rate of contraction with isoprenaline (%)

	Before propanolol	After propanolol
Expt. 3	−24	−4
Expt. 4	−42	−4
Average	−33	−4

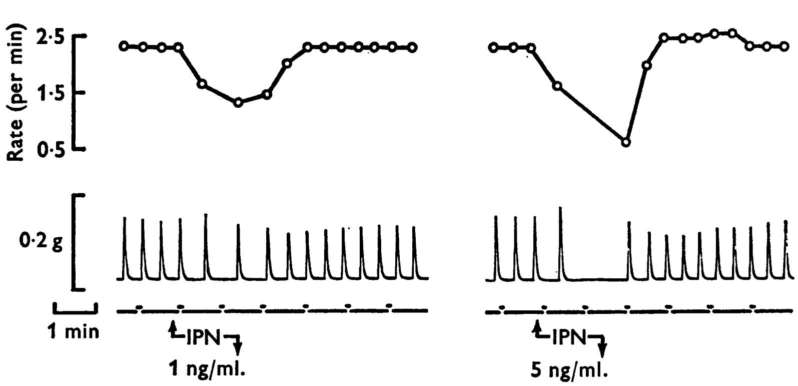

Fig. 7. The effect of isoprenaline on the rate and strength of contraction of bovine lymphatics. Isoprenaline (IPN) caused slowing and slight weakening of the contractions.

Effect of acetylcholine

Fig. 8 shows the effect of acetylcholine on spontaneous activity. On the left, a 1 ng/ml. dose has no effect other than minor disruption of rate. Increasing the concentration to 100 ng/ml., on the right, again only caused minor changes in rhythm. Three vessels were tried and all were insensitive to acetylcholine at these doses.

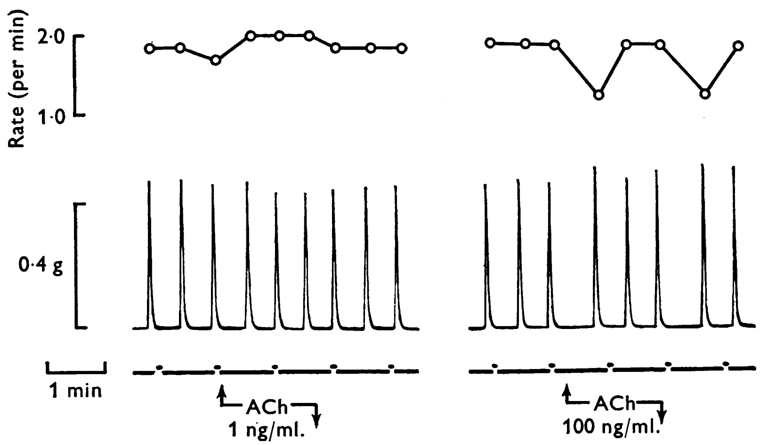

Fig. 8. The effect of acetylcholine on the rate and strength of contraction of bovine lymphatics. The effects of even large doses of acetylcholine (ACh) were small and irregular.

DISCUSSION

Spontaneous activity

It appears that spontaneous activity is a normal feature of bovine mesenteric lymphatics since it was present in the majority of vessels. Its absence in nine vessels could have been due to damage to the tissue during dissection.

This activity, in the presence of valves which are frequent in lymphatics, would serve as an intrinsic mechanism for lymph transport (Hall, Morris & Woolley, 1964). In this respect, regular activity with intervals between contractions would be more efficient than the irregular types. On these grounds, it is suspected that irregular activity is not a normal characteristic of these lymphatics. There are several other reasons for believing this. Irregular activity was observed less often, occurring spontaneously in thirteen of the thirty vessels. It generally took longer to develop, lasted only a short time, and may have been a symptom of deterioration. This latter statement is supported by the observation that the regular activity in a vessel became irregular in rate or amplitude for a period before it ceased to be active. Also, a lymphatic which was non-active or had just ceased to be active could be stimulated to a period of irregular contractions by application of noradrenaline or an electrical stimulus. Regular activity, on the other hand, developed soon after installation of the vessels in the organ bath and lasted for longer periods, up to 8 hr in one case.

Effect of catecholamines

The fact that the effect of noradrenaline could be blocked by phentolamine suggests that the chief effect of α-receptor stimulation in lymphatic muscle is to increase its rate of beating. Thus transport of lymph could be speeded by α-receptor stimulation if there were not a corresponding reduction in the strength of contraction. However, the very weak contractions associated with the high frequencies after large doses of noradrenaline would be quite ineffective in lymph transport.

The increase in the rate of contractions caused by noradrenaline was associated with a decrease in their amplitude. This was also true of spontaneous increases in rate. To see if the reduction in amplitude with noradrenaline could be explained solely by the accompanying rate changes, regression equations for rate on amplitude were calculated for control observations on each lymphatic. These were used to predict the reduction in amplitude which would result from a spontaneous increase in rate. In all cases, the reduction in amplitude with noradrenaline was not as great as would have been expected from the rate changes and in twelve of the fourteen cases this difference was significant (Table 2). These results suggest that noradrenaline increases the strength as well as the rate of lymphatic contraction, i.e. it has a positive inotropic as well as positive chronotropic effect.

The slowing caused by isoprenaline, and its blockade by propranolol indicates that β-receptor stimulation reduces the frequency of contraction. The decrease in the rate of contractions with isoprenaline was associated with a slight reduction in their amplitude. Again this was true of spontaneous decreases in rate. To see if this reduction in amplitude could be explained solely by the rate changes, regression equations for rate on amplitude were worked out for the control periods of the isoprenaline experiments. In all but one case isoprenaline decreased rate to lower values than occurred in control periods. However, in the one case where the rate changes with isoprenaline fitted into the control range of values, the decrease in amplitude with isoprenaline was greater than would have been expected with a spontaneous decrease in rate (Table 2). This suggests that isoprenaline decreases the strength as well as the rate of contraction in lymphatics.

TABLE 2. A comparison of the observed amplitudes of contractions in lymphatics exposed to noradrenaline (NOR) and isoprenaline (IPN) with those predicted from regression equations based on data obtained under control conditions. In all cases with noradrenaline the observed values were greater than the predicted values and in twelve out of fourteen the differences were significant (s) at the 1 in 20 level. In the experiment with isoprenaline the observed value was significantly less than the predicted value

Experiment no.	Drug	Dose (μg/ml.)	Expected amplitude (g)	Observed amplitude (g)	Difference (%)	Significance
1	NOR	1	0·39	0·42	+8	s
2	NOR	1	0·12	0·14	+10	n.s.
3	NOR	1	0·18	0·21	+16	s
4	NOR	1	0·11	0·12	+13	s
5	NOR	1	0·11	0·11	+1	n.s.
6	NOR	5	0·08	0·10	+18	s
7	NOR	5	0·11	0·12	+7	s
8	NOR	10	0·09	0·10	+14	s
9	NOR	10	0·14	0·18	+23	s
10	NOR	10	0·08	0·09	+20	s
11	NOR	10	0·10	0·12	+13	s
12	NOR	20	0·06	0·07	+15	s
13	NOR	50	0·16	0·20	+28	s
14	NOR	50	0·14	0·19	+35	s
15	IPN	1	0·13	0·11	−12	s

Innervation of lymphatics

Several investigations have reported changes in lymphatic vessels when appropriate nerves were stimulated. These are reviewed by Browse (1968). However, Smith (1949) found no alteration in the contractile rate of popliteal lymphatics of rats, mice or guinea-pigs after section or electrical stimulation of the femoral and sciatic nerves.

In the experiments described in this paper, the responses to catecholamines and acetylcholine would suggest that if bovine mesenteric lymphatics are innervated, the nerves would be adrenergic rather than cholinergic.

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