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Responses of the pig isolated renal artery to transmural electrical stimulation and drugs

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1 Pig isolated renal arteries contract in response to addition of noradrenaline, 5-hydroxytryptamine, histamine, angiotensin II, vasopressin and carbachol, whereas cholecystokinin, adenosine, and inosine produce relaxation.

2 Transmural stimulation of the tissue causes contraction of circular muscle in the arterial wall which produces apparent elongation of the vessel.

3 The effects of transmural stimulation are partially blocked by prazosin and potentiated by propranolol, indicating that noradrenaline acts through both α- and β-adrenoceptors.

4 Guanethidine (10^{-5}M) reduces the size of responses to transmural stimulation in the presence of both prazosin (10^{-6}M) and propranolol (10^{-7}M).

5 Both saralasin (10^{-7}M), and desensitization of angiotensin II receptors by prolonged contact with the peptide, produced a reduction in response to transmural stimulation, indicating that angiotensin II may be involved in neurotransmission. This effect was blocked by tetrodotoxin.

6 Transmural stimulation produces relaxation of renal arteries in the presence of maximal doses of saralasin, prazosin, and propranolol, suggesting that a third unidentified substance is also released from autonomic nerves.

Introduction

Transmural electrical stimulation of the ileum, vas deferens and various vascular tissues has provided important information about the functions of autonomic neurotransmitters found in their walls (Paton, 1955; Paton & Vane, 1963; Burnstock *et al.*, 1966: Hughes & Vane, 1967).

We have investigated the reactions of the pig isolated renal artery to both drugs and transmural stimulation. The results show that this tissue contains adrenergic nerves and a non-adrenergic system which appears to be mediated in part by angiotensin II.

Methods

Pig renal arteries were collected fresh from the local slaughter house. For experiments in which drug responses were tested, arteries were cut spirally and suspended vertically in a 50 ml organ bath. For transmural stimulation they were tied over a platinum electrode and surrounded by a platinum wire spiral. The organ bath was maintained at 35°C, and the Krebs solution bubbled with 95% O_2 plus 5% CO_2. Arterial movements were measured with an isotonic transducer (S.R.I. Ltd, Edenbridge, Kent. No. 7042) and recorded on a Heathkit potentiometric recorder (EU 205-22). Electrical stimulation of the arteries was produced by a Nihon Koden MSE-3R stimulator; rectangular pulses lasting 1 ms at maximum output (200 V) were administered for 30 s at various frequencies (5–50 Hz).

Krebs solution had the following composition (mM): NaCl 124, KCl 5, $NaHCO_3$ 26, $CaCl_2$ 0.8, $MgCl_2$ 1.3, KH_2PO_4 1.4 and glucose 10; pH 7.4.

Data analysis

Results are given as means ± standard error of the mean (s.e.mean). Responses to transmural stimulation were compared using Student's *t* test.

Drugs

Agonists: (±)-noradrenaline, angiotensin II, 5-hydroxytryptamine, adenosine, inosine, and arginine-8 vasopressin were obtained from Sigma; cholecystokinin–8 a gift from Dr R. Hill, Parke-Davis Ltd. Antagonists: mepyramine, indomethacin, saralasin

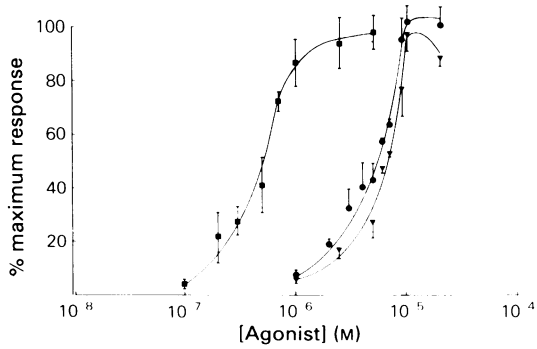

Figure 1 Responses of spirally cut pig renal arteries to angiotensin II (■——■), noradrenaline (●——●) and histamine (▼——▼) as percentages of maximal responses. Absolute maximal tension responses to histamine and noradrenaline were not significantly different, whereas the maximal response to angiotensin II was about 40% greater. Responses given as means with vertical lines showing s.e.means.

and (±)-propranolol were obtained from Sigma; guanethidine from Ciba Ltd; prazosin was a gift from Dr J.A. Gill, Pfizer Ltd, Sandwich, Kent; captopril a gift from Dr R.L. Harding, E.R. Squibb & sons, Hounslow, Middlesex; tetrodotoxin a gift from Dr G. Henderson.

Results

Effects of drugs

Noradrenaline, 5-hydroxytryptamine (5-HT), histamine, angiotensin II, vasopressin, and carbachol contracted spirally cut arteries – see log dose-response curves in Figure 1. Cholecystokinin produced marked and long lasting relaxations while adenosine and inosine produced short lasting relaxations. Angiotensin II, as found by Hughes & Vane (1967) in rabbit portal vein, produces tachyphylaxis, but it was found with pig renal arteries that doses administered at 30 min intervals produced consistent responses.

Prazosin produced a parallel shift to the right of the log dose-response curve to noradrenaline, as did mepyramine to histamine, saralasin to angiotensin II, whereas indomethacin (10^{-6} M) produced non-competitive blockade of responses to 5-HT. Propranolol (10^{-7} M) had no effect on contractions induced by addition of noradrenaline.

Effect of transmural stimulation

Pig renal arteries responded to transmural stimulation with a rapid relaxation which recovered slowly (10–15 min). When transverse and longitudinal strips were subjected to field stimulation, it was clear that such stimulation produced contraction of circular muscles only, so that the relaxation seen in intact arteries was produced by contraction of circular muscle increasing the length of the artery. Responses to transmural stimulation though not to exogenous noradrenaline, were completely abolished by 2×10^{-6} M tetrodotoxin.

Table 1 Effect of prazosin on responses to transmural stimulation

Stimulus frequency (Hz)	*Relaxation produced (% of maximum)*	
	Control	*Prazosin* (10^{-6} M)
10	21.4 ± 5.2	12.6 ± 2.41*
20	42.9 ± 8.6	17.4 ± 3.4*
30	53.4 ± 10.3	27.6 ± 4.2*
40	100.0 ± 0.0	32.0 ± 4.6*

Relaxation responses were maximal at 40 Hz. Results are expressed as means ± s.e.mean from four separate renal arteries. * $P < 0.05$ vs control response.

Effects of adrenoceptor blockers

To try and identify the neurotransmitters released by transmural stimulation it was necessary to use spirally cut arteries to determine concentrations of specific antagonists which would block completely the effects of agonists suspected of being present. The ED_{50} of prazosin in blocking the effect of added noradrenaline in pig renal artery was found to be 3×10^{-8} M, and a concentration (5×10^{-6} M) in excess of that required for maximal inhibition of the effects of noradrenaline reduced the relaxations produced by transmural stimulation by about half (Table 1).

Table 2 Effect of propranolol on responses to transmural stimulation

Stimulus frequency (Hz)	*Relaxation produced (% of maximum)*	
	Control	*Propranolol* (10^{-6} M)
20	25.3 ± 3.0 ($n = 3$)	50.7 ± 0.3 ($n = 3$)*
20	25.0 ± 6.8 ($n = 3$)	66.4 ± 4.2 ($n = 3$)*
40	100.0 ± 12.3 ($n = 2$)	107.8 ± 12.3 ($n = 2$)
40	100.0 ± 2.4 ($n = 2$)	95.4 ± 1.8 ($n = 2$)*

Relaxation responses were maximal at 40 Hz. * $P < 0.05$ vs control response, results from 2 separate preparations.

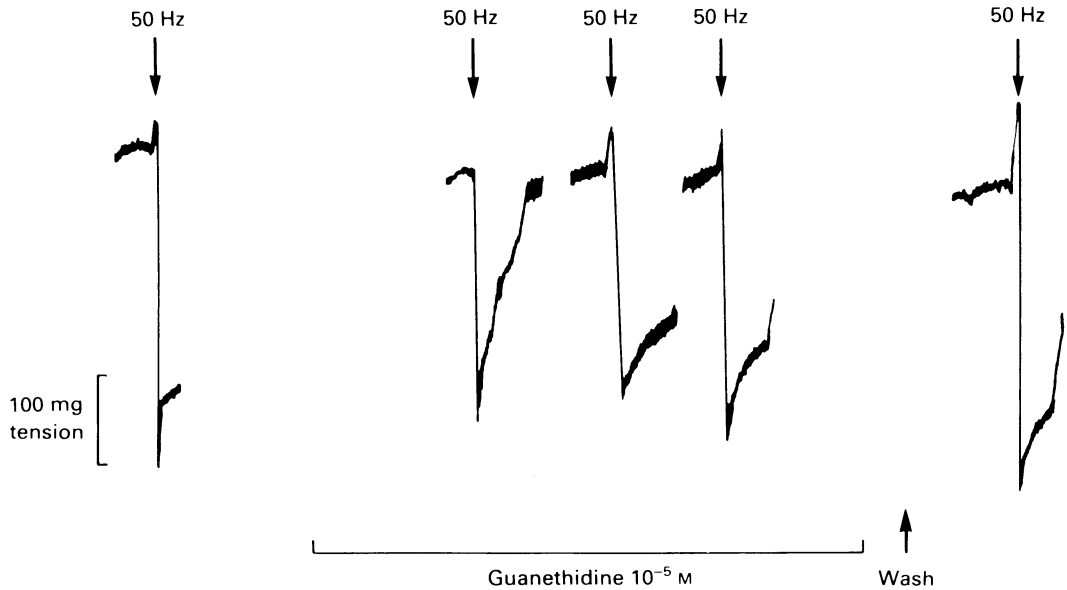

Figure 2 Inhibitory effect of guanethidine (10^{-5} M) on transmural stimulation of renal arteries in the presence of prazosin (10^{-6} M) and propranolol (10^{-7} M) throughout. Means ± s.e.mean of control group were 99.8 ± 3.63 and in the presence of guanethidine 51.9 ± 15.2; $P < 0.001$ (results expressed as % control response to stimulation at 40 Hz, from 4 different preparations). The presence of guanethidine is indicated by the horizontal line. Upper arrows indicate electrical stimulation, at 50 Hz, lower arrow washout of guanethidine. Guanethidine was present for 25 min, the washout was followed by a further 30 min before transmural stimulation was repeated.

Although propranolol (10^{-7} M) had no effect on the responses to noradrenaline when added to spirally cut arteries, it potentiated the relaxation produced by transmural stimulation at 20 Hz, though not at 40 Hz (Table 2). This propranolol concentration was higher than that used by Davis (1970) to achieve maximum blockade of β-receptor mediated effects, but below that required for local anaesthetic actions. When renal arteries were stimulated transmurally in the presence of maximal doses of both prazosin (10^{-6} M) and propranolol (10^{-7} M), it was found that the addition of guanethidine (10^{-5} M) further reduced the relaxation responses by between a third and a half (Figure 2).

Table 3 The effect of saralasin on transmural stimulation of the pig artery

Stimulus frequency (Hz)	*Relaxation produced (% of maximum)*	
	Control	*Saralasin* (10^{-7} M)
20	46.6 ± 4.3	28.0 ± 2.0*
40	100.0 ± 11.2	87.1 ± 12.0

Relaxation responses were recorded in the presence of propranolol 10^{-7} M and prazosin 10^{-6} M. Control responses to stimulation at 40 Hz are taken as 100%, other results are expressed as a percentage response relative to this. Values shown are means ± s.e.means of 4 observations from 4 different preparations; $P < 0.05$ vs control.

Effects of drugs acting on the renin-angiotensin system

To investigate the non-adrenergic component of the renal artery innervation, the next series of experiments

Table 4 Desensitization of angiotensin II receptors

Stimulus frequency (Hz)	*Control relaxation*	n	*Relaxation after desensitization*	n	P
20	66.8 ± 3.9	3	33.7 ± 4.1	2	<0.02
40	100.0 ± 12.3	2	88.7 ± 0.5	2	n.s.
20	88.4 ± 3.9	3	22.0 ± 2.2	3	<0.001
40	100.0 ± 11.6	2	64.6 ± 3.0	3	<0.05
20	49.0 ± 1.0	4	32.1 ± 3.1	2	<0.005
40	100.0 ± 11.1	4	48.4 ± 11.8	2	n.s.

Results are expressed as a percentage of the control responses to stimulation at 40 Hz. Values shown are means ± s.e.means from 3 separate experiments. n = number of observations on each artery, P = probability of difference using Student's t test.

were performed using saralasin (Sar[1], Ala[8] angiotensin II) a competitive inhibitor of angiotensin II. The results are shown in Table 3. In addition, responses of pig renal arteries were compared before and after contact with angiotensin II (10^{-6} M) for 30 min to desensitize angiotensin receptors. The bathing solution contained propranolol (10^{-7} M) and prazosin (10^{-6} M). The results of three separate experiments are shown in Table 4. It was demonstrated that recovery from desensitization occurred if angiotensin II was washed out and the preparation left for 1 h before transmural stimulation was repeated.

Discussion

Transmural stimulation of smooth muscular tissues and organs from different animals shows that noradrenaline is only one of two or more transmitters responsible for the smooth muscular activity, (Hughes & Vane, 1967; Ambache & Aboo Zar, 1971; McGrath, 1978; Burnstock, 1981). Although large numbers of agonists produce contractions of smooth muscle in renal arteries, the effects of transmural stimulation are mediated substantially by noradrenaline which contracts smooth muscle by stimulation of α-adrenoceptors, but may also affect γ-adrenoceptors (Hirst & Neild, 1981). The fact that propranolol was without effect when noradrenaline was added to spirally cut preparations, but potentiated the responses to transmural stimulation suggests that it may have affected neurotransmitter release.

Our results indicate that angiotensin II is released and contributes to arterial wall smooth muscle contraction after transmural stimulation. As it is blocked by tetrodotoxin this effect is neurogenic; however, it is still seen in the presence of maximal doses of prazosin and propranolol, which makes it unlikely that the effect is mediated entirely by noradrenaline release acting through β-adrenoceptors to release renin. Celio & Inagami (1981), have detected angiotensin II together with renin in juxtaglomerular cells in kidney by immunohistochemical methods, though the mode of release of this angiotensin is unknown. Guanethidine reduced the relaxation response caused by transmural stimulation in the presence of both prazosin and propranolol, a finding in accord with the idea that the effect of noradrenaline released from neurones is not entirely blocked by α- and β-receptor antagonists (Hirst & Neild, 1981).

The evidence that autonomic nerves release at least two neurotransmitter substances in tissues such as blood vessels and vas deferens relies not only on the failure of pharmacological antagonists such as prazosin to block the effects of nervous stimulation completely, but also on the fact that the time relationship between stimulation and response and the nature of the electrical responses themselves differ. Further, in vas deferens the two ends of the tissue differ in their responses to nerve stimulation (McGrath, 1978). Our findings suggest that in the renal artery at least, the smooth muscle responses to transmural stimulation are mediated by three transmitters, noradrenaline, angiotensin II and a third as yet unidentified compound.

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