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THE RESPONSE OF THE ISOLATED DUCTUS ARTERIOSUS TO TRANSMURAL STIMULATION AND DRUGS

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1 Responses of isolated ductus arteriosus preparations from near term guinea-pigs and lambs to transmural electrical stimulation and drugs were studied in a low oxygen medium (Po_2 19 to 28 mmHg).

2 Acetylcholine and noradrenaline contracted both vessels in a dose-dependent manner, their threshold being between 10^{-8} and 10^{-7} M. Transmural stimulation (pulse width 0.2 to 0.6 ms, typically 20 Hz) also contracted the vessels.

3 Atropine and phentolamine or dibenzyline selectively blocked responses to acetylcholine and noradrenaline, respectively.

4 In the guinea-pig ductus, part of the response to transmural stimulation was due to activation of intrinsic adrenergic nerves since the responses were reduced by α-adrenoceptor antagonists, bretylium or prior reserpine treatment, but not by atropine. The response of the lamb ductus to transmural stimulation varied greatly in magnitude and was inconsistently affected by α-adrenoceptor blocking drugs.

5 There was no evidence that transmural stimulation activated cholinergic nerves in either species.

6 After inactivation of α-adrenoceptors with dibenzyline, noradrenaline caused a β-adrenoceptor-mediated relaxation. Both this effect and isoprenaline-mediated relaxation were blocked by propranolol. β-Adrenoceptor activity was more prominent in the ductus of the guinea-pig than of the lamb.

7 Raising the Po_2 from 19–28 to 92–98 mmHg increased the response of the guinea-pig ductus to transmural stimulation suggesting that, in this species, physiological elevation of oxygen tension at birth may increase transmitter release from intrinsic adrenergic nerves. Whether this mechanism would contribute to ductus closure remains an open question.

8 We postulate that β-adrenoceptor-mediated relaxation has a role in maintaining ductus patency in the guinea-pig foetus.

Introduction

Elevation in blood oxygen tension at birth is the prime stimulus to closure of the ductus arteriosus (cf. Heymann & Rudolph, 1975). While some investigators propose that oxygen acts directly on ductal muscle (Kovalčík, Kriška & Doležel, 1969; Fay, 1971), others have suggested that a humoral agent mediates the oxygen response (Born, Dawes, Mott & Rennick, 1956; Melmon, Cline, Hughes & Nies, 1968; Hörnblad, Boréus & Larsson, 1970; Oberhänsli-Weiss, Heymann, Rudolph & Melmon, 1972). Noradrenaline and acetylcholine have been most often considered for this role because they both constrict the ductus (Born *et al.*, 1956; Kovalčík, 1963; Boréus, Malmfors, McMurphy & Olson, 1969; Aronson, Gennser, Owman & Sjöberg, 1970; McMurphy, Heymann, Rudolph & Melmon, 1972) and they may also be released from intramural nerve fibres (Boréus *et al.*, 1969; Kovalčík *et al.*, 1969; Aronson *et al.*, 1970; Ikeda, 1970; Silva & Ikeda, 1971; Ikeda, Sonnenschein & Masuoka, 1972). However, experimental results are conflicting. Findings implicating neuronally released acetylcholine in the oxygen-induced constriction of the lamb ductus (Oberhänsli-Weiss *et al.*, 1972) were not confirmed (Perin, Coceani & Olley, 1974; Noel & Cassin, 1976). Treatment of full term pregnant guinea-pigs with dibenzyline shortly before caesarean delivery significantly delays ductus closure in the neonates (Hörnblad *et al.*, 1970) implying a role for catecholamines acting on α-adrenoceptors. In contrast, the isolated ductus from the guinea-pig and lamb remains responsive to oxygen after treatment

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with α-blockers (Kovalčík, 1963; Oberhänsli-Weiss *et al.*, 1972) or monoamine depletion (Kovalčík *et al.*, 1969). An intermediary role for noradrenaline and acetylcholine in the oxygen response therefore seems unlikely, although either, or both, agents could facilitate the action of oxygen (McMurphy *et al.*, 1972; Ikeda, Rubenstein & Sonnenschein, 1973).

With this in view, we have studied the response of the isolated ductus arteriosus to transmural electrical stimulation and to some drugs acting on the autonomic nervous system. The aim was to add pharmacological evidence to existing histochemical and ultrastructural data on the nature of autonomic innervation and to characterize the receptors mediating responses to neurotransmitters. An additional objective was to compare responses in the lamb and guinea-pig. Since the latter species lacks a prostaglandin-mediated relaxing mechanism (cf. Coceani, Olley, Bishai, Bodach & White, 1978), which is so important in the lamb, it is possible that autonomic mechanisms may also differ in these two species.

Methods

General procedure

Guinea-pig Pregnant guinea-pigs at term (>60 days) were anaesthetized with intraperitoneal urethane (2 g/kg) and their foetuses were delivered by caesarean section into a low-oxygen environment (P_{O_2} 10 mmHg). With the umbilical cord intact, foetuses underwent a left thoracotomy; a block of tissue, including the ductus arteriosus and a section of the adjacent large vessels, was dissected out and transferred to a dish containing Krebs solution gassed with a mixture of 2.5% O_2 and 5% CO_2 in N_2. A ring of ductus arteriosus was prepared and then suspended between platinum hooks in a 20 ml organ bath. The lower hook was part of a stationary glass rod while the upper hook was connected to a force-displacement transducer (Grass FT-03C) by silk. The preparation was positioned between two parallel glass-shielded platinum wires for transmural stimulation and the initial tension was adjusted to 0.4 to 0.5 g. The weight of the foetuses (59 to 126 g) confirmed (cf. Draper, 1920) that the animals were within a few days of delivery.

Lamb The procedures for caesarean delivery of near term foetal lambs and preparation of circular strips of ductus arteriosus (mid-portion) have been published (Coceani & Olley, 1973). Strips, about 1 mm wide and 9 mm long, were mounted between two platinum electrodes as already described, but the Krebs solution was pre-equilibrated with 5% CO_2 in N_2. After stretching the tissue to a passive tension of about 1.5 g, the Krebs solution was changed to one pre-equilibrated with 2.5% O_2 and 5% CO_2 in N_2.

With both preparations, recording was isometric and was displayed on a Grass polygraph. The organ bath was supplied from several reservoirs and a system of three-way valves allowed a rapid change from one perfusion fluid to another. The perfusion rate was approximately 2 ml/min and the fluid temperature was 37°C. Both the reservoir and organ bath were continuously bubbled with the required gas mixture. Unless otherwise specified, a mixture of 2.5% O_2 and 5% CO_2 in N_2 was used. This mixture gave a P_{O_2} of between 19 and 28 mmHg, which equals the physiological P_{O_2} and is below the threshold for the contractile effect of oxygen on ductal muscle (Fay, 1971; Oberhänsli-Weiss *et al.*, 1972). The oxygen content of the medium was measured with an Instrumentation Laboratory gas analyzer. Ambient light was constant throughout the experiment.

Solutions and drugs

The Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1, $MgSO_4$ 0.9, dextrose 11.1 and $NaHCO_3$ 25. Potassium-Krebs solution (55 mM) was prepared by substituting NaCl with an equimolar amount of KCl. The pH of the solution was 7.4 after equilibration with gas mixtures containing 5% CO_2.

The following drugs were used: acetylcholine chloride (Calbiochem), atropine (Sigma), bretylium tosylate (Burroughs Wellcome), dibenzyline hydrochloride (Smith, Kline and French), (±)-isoprenaline hydrochloride (Sigma), (−)-noradrenaline bitartrate (Sigma), phentolamine hydrochloride (Ciba), (±)-propranolol hydrochloride (Ayerst), reserpine (Sigma and Ciba).

Stock solutions of catecholamines (3 to 10 mg/ml) containing 1 mg/ml ascorbic acid were stored at −20°C and thawed on the day of the experiment. These solutions were diluted with saline and were kept on ice during use. Other drug solutions were prepared as required. Compounds were tested in sequential or, sometimes, in cumulative doses (3 to 10 fold increments in 20 to 100 µl volumes). In the latter case, the total sample volume never exceeded 5% of the organ bath capacity. Doses of all drugs are given in molar concentrations and refer to their final concentration in the bath.

Reserpine treatment

Pregnant guinea-pigs were treated with reserpine 24 h before caesarean delivery and preparation of the ductus. The drug was given intraperitoneally (1.5 mg/kg) as a fine suspension of 3 mg/ml in propylene

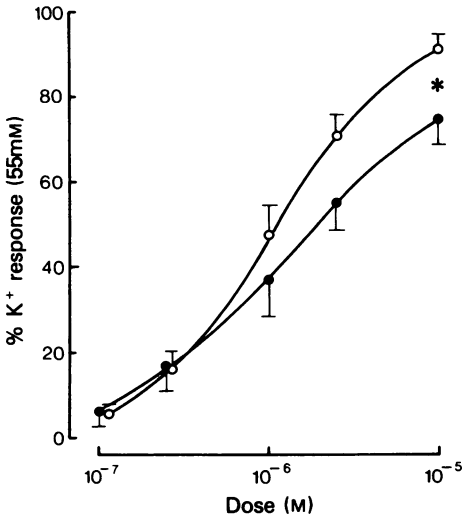

Figure 1 Effects of acetylcholine (●) and noradrenaline (○) on muscle tension of the guinea-pig ductus arteriosus. Tension developed is expressed as a percentage of the contractile response to 55 mM K^+ (1.76 ± 0.1 g). Compounds were tested in sequential ($n = 4$) or cumulative ($n = 1$) doses. $*P < 0.05$ (paired t test).

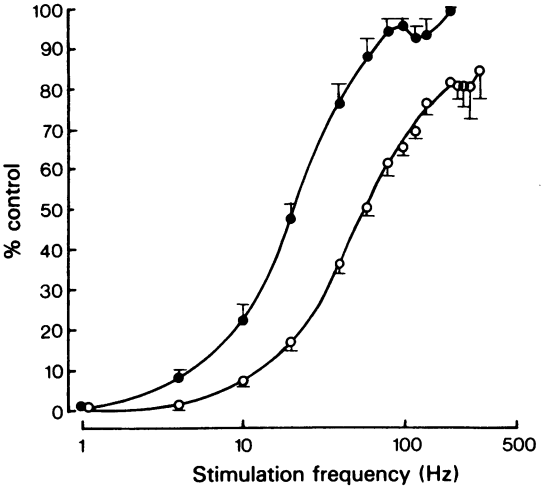

Figure 2 Effects of transmural electrical stimulation at different frequencies (75 V, 0.5 ms for 20 s) on the guinea-pig ductus arteriosus before (●) and after (○) treatment with dibenzyline (5×10^{-7} M). Dibenzyline was added to the bath, allowed 10 min contact and washed out. Responses are expressed as a percentage of the maximal response to stimulation before dibenzyline. Differences between the curves are significant at the 2% level or better (paired t test; $n = 5$) at frequencies from 4 Hz upwards.

glycol or subcutaneously (0.5 mg/kg) as an aqueous solution (Serpasil, Ciba).

Transmural stimulation

The ductus was stimulated with rectangular pulses of 0.2 to 0.6 ms duration and maximal voltage output (70 to 100 V) allowed by the stimulator (Grass mod. S88). Preliminary experiments showed that ductal responses declined in time when a stimulator assembly delivering pulses of greater strength (cf. Vanhoutte, Clement & Leusen, 1967) was used. Pulses were applied at 20 Hz for periods of 5 to 40 s, and the interval between trains was adjusted to allow for full recovery. Different frequencies when used are indicated in the text.

Analysis of responses

Effects of contractile agents and transmural stimulation were measured by the increase in tension from the basal tension (Δ tension). Basal tension, which varied depending on the animal preparation and the Po_2 of the medium (see Results), is given after correction for the tension applied at the start of the experiment. To determine the effects of relaxant agents, tissues were contracted by exposure to oxygen (guinea-pig and lamb) or to indomethacin 2.8×10^{-6} M (lamb). Data are expressed as the mean ± s.e.

Statistical analysis of unpaired data, paired data, slopes and differences between slopes has been made using Student's t test.

Results

Responses to noradrenaline, acetylcholine and transmural stimulation

Guinea-pig Ductal rings ($n = 58$) reached steady baseline in 1 to 2 h. Preparations had little tone (<0.3 g) or spontaneous activity in the low oxygen medium (Po_2 19 to 28 mmHg); however, the muscles contracted upon exposure to higher oxygen levels and a maximal or near-maximal response (mean, 1.9 g; range, 1.3 to 2.8 g) was obtained at a Po_2 of 217 to 230 mmHg. At a Po_2 of 92 to 98 mmHg, the peak contractile tension ranged between 0.4 and 1 g (mean, 0.7 g).

Acetylcholine and noradrenaline contracted the ductus in a dose-dependent manner. While the threshold concentration was similar for both compounds, noradrenaline produced relatively larger responses at higher concentrations (Figure 1). Likewise, noradrenaline tended to be more effective than acetylcholine (86 ± 7 vs. 67 ± 8% of K^+ response at 10^{-5} M,

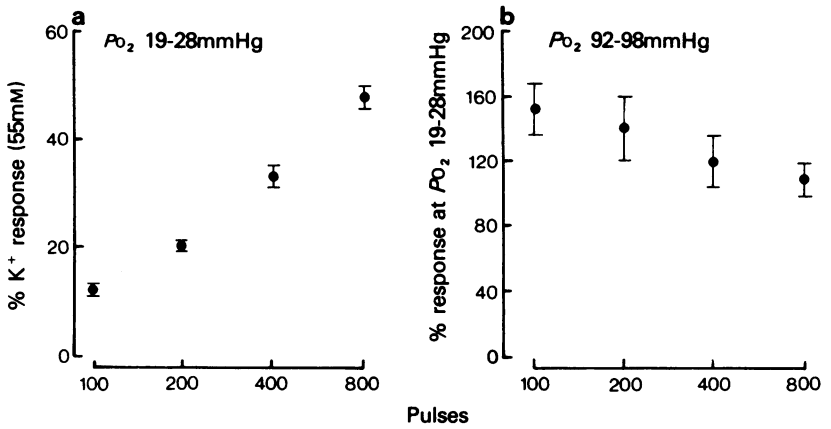

Figure 3 Guinea-pig ductus arteriosus response to transmural stimulation (70–75 V, 0.5 ms, 20 Hz). (a) Increase in the amplitude of responses with the number of pulses applied ($n = 22$). Reference K^+ (55 mM) response, 1.99 ± 0.09 g. (b) Oxygen-induced enhancement of contractile responses. A significant negative correlation was found between the oxygen effect and the number of pulses applied ($P < 0.01$; $n = 4$).

$n = 3$) on tissues partially contracted by oxygen (Po_2 92 to 98 mmHg). With either compound, responses were immediate in onset and showed no evidence of tachyphylaxis.

Transmural electrical stimulation of the ductus evoked a prompt contraction the magnitude of which increased with pulse frequency up to 100 Hz (Figure 2). However, the susceptibility of this response to dibenzyline varied with the frequency of stimulation. As shown in Figure 2, with increasing frequencies a progressively smaller fraction of the response was blocked by dibenzyline; moreover, dibenzyline-treated tissues required 200 Hz stimuli for maximal activation. A frequency of 20 Hz producing responses with a relatively small dibenzyline-resistant component was therefore used in all subsequent experiments. Contractions also increased in size with the number of pulses delivered at 20 Hz (Figure 3a). However, shorter trains became more effective at higher Po_2 (Figure 3b).

Lamb The lamb ductus required 1 to 5 h to equilibrate at low Po_2 (19 to 24 mmHg). When first set up, resting tension gradually increased to an average value of 2.1 g (range, 0.7 to 4.2 g; $n = 21$). Tension then fell in all but two experiments and stabilized at or above the original baseline (mean, 0.9 g; range, 0.3 to 2 g). The tissue contracted further when exposed to a higher Po_2, and the maximal tension developed was 3.5 g (range, 1.8 to 5.4 g) and 5.5 g (range, 3.8 to 7.6 g) respectively at 92 to 98 and 595 to 689 mmHg.

Drugs and electrical stimulation were applied after the preparation had stabilized. Both acetylcholine and noradrenaline caused a dose-related contraction of the muscle. Unlike the guinea-pig ductus, the lamb ductus was more sensitive to acetylcholine than to noradrenaline, their threshold concentrations being respectively 10 to 25 nM and 25 to 100 nM. The relative efficacy of the two compounds, however, could not be determined exactly because dose-response curves were different. While responses to acetylcholine increased with concentrations up to 1 to 2.5×10^{-6} M (max. Δ tension, 2.58 ± 0.18 g; $n = 3$) and declined thereafter, responses to noradrenaline were still submaximal at 2.5×10^{-6} M (Δ tension, 1.66 ± 0.53 g; $n = 3$). In two additional experiments, several responses to each drug at 10^{-6} M were compared on the same preparation and acetylcholine proved to be consistently more potent.

Electrically-induced contractions increased in amplitude with the frequency of stimulation (1 to 40 Hz) or with the number of pulses delivered at 20 Hz. Increases in tension in response to 800 pulses (20 Hz) varied from 0.3 to 2.7 g (mean, 1 g; $n = 17$).

Neither tissue contracted to single transmural shocks, nor did a low-frequency stimulation (0.5 to 1 Hz) cause relaxation. In the lamb, contractile responses to 20 Hz trains were sometimes followed by a small relaxation.

Effects of atropine, phentolamine and dibenzyline

Atropine (10^{-8} M) or phentolamine (10^{-8} or 10^{-6} M) were included in the Krebs solution bathing the ductus. Dibenzyline (5×10^{-7} M) was added directly to the bath, allowed 10 min contact with the tissue and washed out. The latter procedure was found to

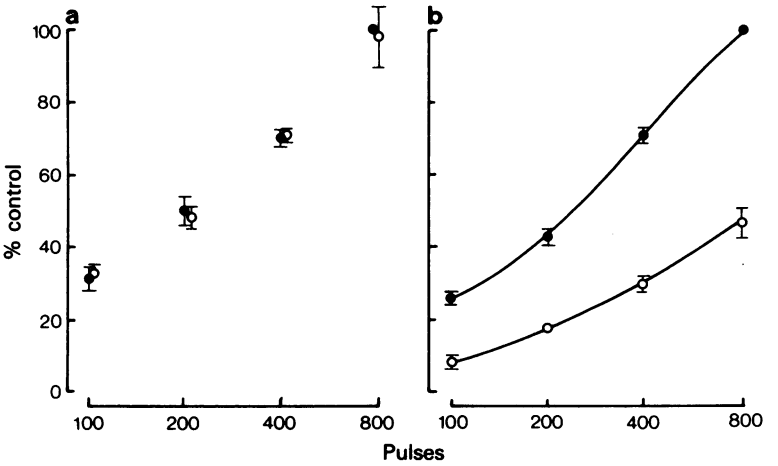

Figure 4 Guinea-pig ductus response to transmural stimulation (75 V, 0.5 ms, 20 Hz) before (●) and after (○) addition of (a) atropine 10^{-8} M ($n = 3$) or (b) phentolamine 10^{-6} M ($n = 9$) to the perfusion fluid. Responses are expressed as a percentage of the maximal response before treatment. Differences between control and phentolamine-treated tissues are significant ($P < 0.001$, paired t test).

inhibit noradrenaline-induced contractions without affecting acetylcholine responses.

Guinea-pig Atropine had no effect on the response to transmural stimulation over the range of pulses used (Figure 4a). Conversely, atropine decreased the contraction to added acetylcholine and the mean dose-ratio for dose-response curves before and during treatment was 27. Phentolamine had an insignificant effect at 10^{-8} M ($n = 3$), but at 10^{-6} M it reduced noradrenaline responses, though to a variable degree (mean dose-ratio, 60; range, 9 to 119), and also reduced the contraction to transmural excitation (Figure 4b). Dibenzyline produced a similar effect on the electrically-induced response (Figure 2). Furthermore, if the ductus had intrinsic tone, dibenzyline converted the contractile effect of noradrenaline to a relaxation (Figure 6). With either of these antagonists, responses to shorter trains were affected more than those to longer trains. In four separate experiments, ductuses were pre-equilibrated at a Po_2 of 92 to 98 mmHg and their response to transmural stimulation was also depressed following treatment with dibenzyline (mean reduction 63% and 50% for trains of 100 and 800 pulses respectively).

Lamb The lamb ductus differed from the guinea-pig ductus in that its contraction to transmural excitation had variable magnitude and was unevenly affected by treatment with dibenzyline. Dibenzyline (5×10^{-7} M) was ineffective in three experiments in which transmural responses were small (0.5 g or less for a 800 pulse train) while it reduced by 55% to 75% ($n = 4$) larger responses. Whether or not effective on electrical stimulation, the drug blocked or reversed the contractile response to added noradrenaline. In all cases, atropine failed to modify responses to transmural stimulation.

With the exception of dibenzyline, antagonists had no effect on the basal tone of either preparation, nor did they alter the contractile response to oxygen. Dibenzyline often produced a modest and rapidly reversible relaxation.

Effects of prior reserpine-treatment on ductal responses

In all ductus rings prepared from reserpine-treated guinea-pigs, transmural responses were reduced by more than 50% compared to untreated ductuses (Figure 5). The residual contraction was unaffected by treatment with either dibenzyline or atropine (Figure 6). In contrast, depending on the dose of reserpine used, responses to noradrenaline remained unchanged (Figure 5) or increased 4 to 5 times ($n = 3$) over control values (Figure 6).

Effects of bretylium

Bretylium (4 to 6×10^{-5} M) produced a progressive decrease in the response of guinea-pig preparations to transmural electrical stimulation ($n = 5$). Maximal reduction was attained in about 60 to 70 min and the residual response varied between 32 and 79% of controls. No further decrease occurred upon treatment with atropine (10^{-7} M). The proportion of the response blocked was inversely related to the frequency

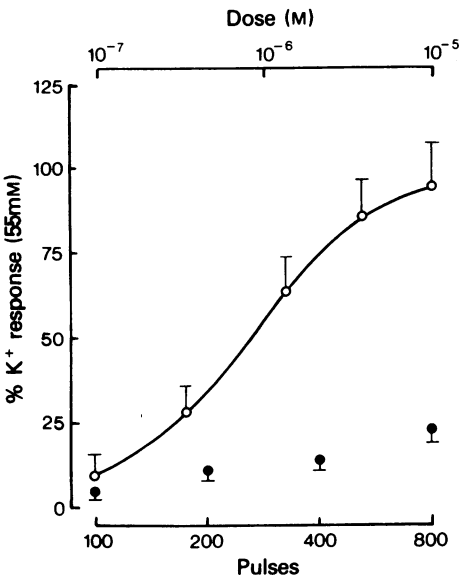

Figure 5 Responses of the ductus arteriosus from reserpine-treated guinea-pigs (0.5 mg/kg s.c.) to transmural stimulation (●) and noradrenaline (○) ($n = 3$). Transmural responses are reduced ($P < 0.02$ when comparing least square fits for these responses and the control responses of Figure 3), while noradrenaline responses are not significantly different from controls. Both responses are expressed as a percentage of the potassium-induced (55 mM) contraction (1.7 ± 0.2 g). Note that the ED_{50} concentration of noradrenaline for treated tissues (0.81 ± 0.27 μM) does not differ from that of controls (0.88 ± 0.16 μM; $n = 11$).

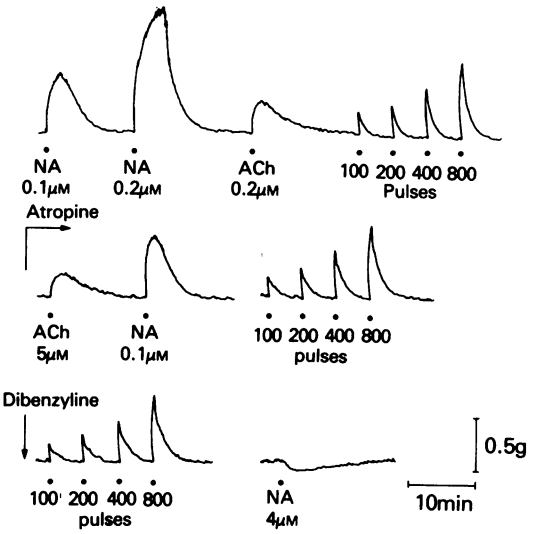

Figure 6 Ductus arteriosus from a guinea-pig treated with a high dose of reserpine (1.5 mg/kg i.p.). Unlike controls, noradrenaline (NA) was more effective than acetylcholine (ACh) at a low dose. Atropine (10^{-7} M) was added to the perfusion fluid, while dibenzyline (5×10^{-7} M) was kept in contact with the tissue for a 6 min period and then washed out. Neither treatment modified the response to transmural electrical stimulation. Note that after treatment with dibenzyline the response to noradrenaline was reversed. In this and subsequent figures, numbers under transmural responses indicate the number of pulses applied.

of stimulation over a range between 5 and 40 Hz and also to the duration of stimulation at 20 Hz (Figure 7). Noradrenaline-induced contractions were either not affected or were potentiated by bretylium. Noradrenaline overcame the blockade of transmural responses caused by bretylium (Figure 8).

Receptors mediating catecholamine responses in the ductus arteriosus

Guinea-pig As shown in Figure 6, contractions of the guinea-pig ductus to noradrenaline were often converted to relaxations following treatment with dibenzyline. In the experiments described below, we showed that this relaxant effect of noradrenaline was due to activation of β-adrenoceptors. Contractile responses were established to noradrenaline; then the tone of the muscle was raised by increasing the Po_2 of the medium (92 to 98, 217 to 230, or 630 to 651 mmHg) and α-adrenoceptors were inactivated with dibenzyline as already described. When the muscle had stabilized, noradrenaline in concentrations higher than those required for α-adrenoceptor activation now caused a relaxation (threshold 0.4 to 4×10^{-5} M; $n = 7$). In the same preparation, isoprenaline was more potent than noradrenaline, its action being dose-dependent in the range 10^{-8} to 10^{-5} M (Figure 9; $n = 4$). Relaxant responses to noradrenaline and isoprenaline were antagonized by propranolol (10^{-7} M). β-Adrenoceptor activity following α-adrenoceptor inactivation was more pronounced in ductuses made supersensitive to catecholamines by prior reserpine-treatment (1.5 mg/kg i.p.). At a concentration of 10^{-6} M, isoprenaline almost completely reversed the tension developed by the reserpine-treated ductus (92%, $n = 2$), whereas it produced only about 40% of the maximal possible relaxation in controls (Figure 9).

Lamb Noradrenaline (1 to 4×10^{-6} M) and isoprenaline (10^{-9} to 10^{-4} M) also relaxed lamb ductus preparations which had been pretreated with dibenzyline ($n = 8$). Effects of both compounds were modest (max. 0.4 g) even at the highest doses and were seen consistently only in tissues with little intrinsic tone (Po_2 22 to 24 mmHg). Ductuses contracted by oxygen

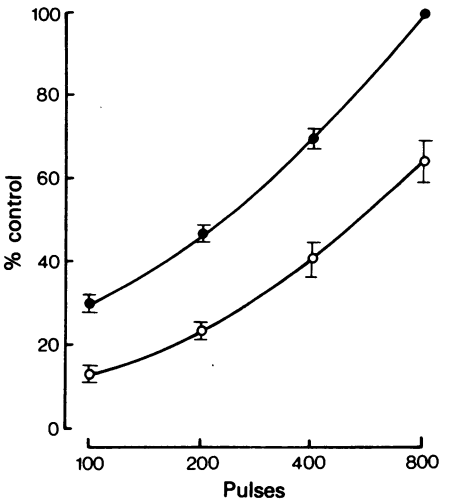

Figure 7 Response of the guinea-pig ductus to transmural stimulation (75–80 V, 0.2–0.5 ms, 20 Hz) before (●) and after (○) addition of bretylium 4×10^{-5} M to the perfusion fluid. Responses are expressed as a percentage of the maximal response before treatment. Differences between control and treated tissues are significant at the 2% level or better (paired t test; $n = 4$).

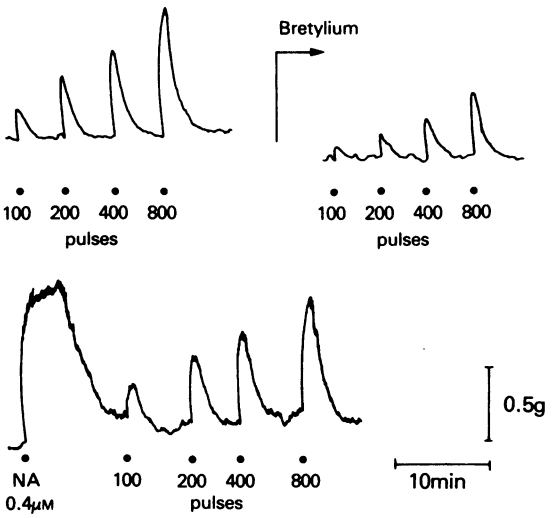

Figure 8 Guinea-pig ductus. Partial blockade of electrical stimulation (60 V, 0.2 ms, 20 Hz) by bretylium and restoration by noradrenaline (NA). Bretylium 6×10^{-5} M was added to the perfusion medium at the arrow.

(Po_2 92 to 98 or 595 to 689 mmHg) or indomethacin (2.8×10^{-6} M) were either unresponsive or failed to respond in a dose-dependent manner. Results were the same regardless of whether drugs were tested first on the non-contracted tissue or directly on the contracted tissue.

Discussion

The present investigation employing transmural electrical stimulation (Paton, 1957) shows that the ductus arteriosus from near term guinea-pigs contains functional adrenergic nerves. This conclusion, which accords with histochemical data (Kovalčík *et al.*, 1969), is supported by the following findings: (a) electrically-evoked contractions were selectively reduced by α-adrenoceptor antagonists and the degree of blockade was inversely related to the frequency of stimulation; (b) prior reserpine-treatment of the animals, although it enhanced responses to exogenous noradrenaline, reduced responses to transmural electrical stimulation; and (c) the adrenergic neurone blocking agent, bretylium also decreased the contraction to transmural stimulation.

While our results provide satisfactory evidence for an adrenergic mechanism in the guinea-pig ductus, they are inconclusive in the lamb. The lamb ductus, unlike that of the guinea-pig, contracted variably to electrical stimulation and its contractile responses were unevenly affected by α-adrenoceptor blockade. A possible reason for this inconsistency is that the level of oxygenation, though it mimicked the foetal condition, may not have been optimal for neurotransmitter release. Indeed, we found in the guinea-pig that short pulse trains, which are likely to favour the activation of nervous tissue, became more effective upon raising the oxygen tension of the medium. Oxygen availability may be an even more important limiting factor with the lamb ductus preparation because of its greater thickness. However, this explanation could not account for the variability of responses which occurred in spite of the fairly uniform size of the preparations. An alternative, and more likely, explanation is that adrenergic fibres are unevenly distributed in the lamb ductus. Consistent with this possibility are biochemical data proving that noradrenaline levels in ductal tissue decrease from the pulmonary to the aortic end of the vessel (Ikeda *et al.*, 1972).

In neither species was evidence obtained to suggest that neuronally released acetylcholine contributes to electrically-induced contractions. This finding argues against the occurrence of functional cholinergic nerves in the vessel wall (Silva & Ikeda, 1971) and contrary to the proposal of Oberhänsli-Weiss et al. (1972), confirms that acetylcholine is not an intermediary in the contractile response of ductus muscle cells to oxygen (cf. Perin *et al.*, 1974; Noel & Cassin, 1976). Acetylcholine, however, is a potent ductal con-

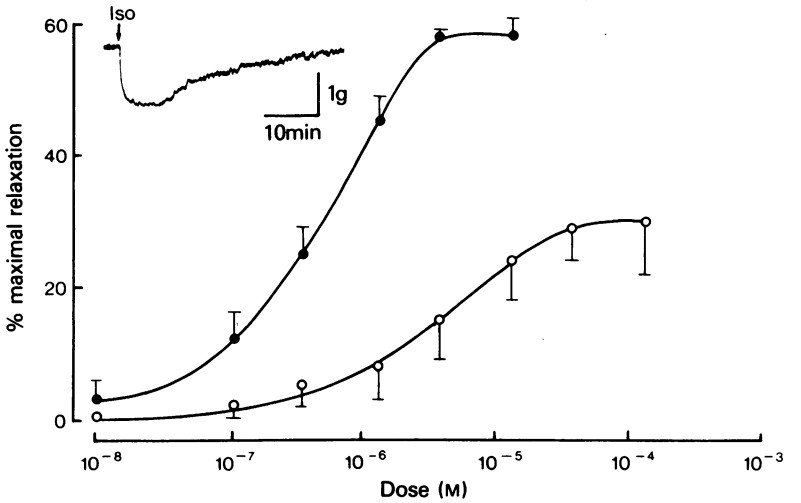

Figure 9 Guinea-pig ductus. Dose-response curves for the relaxant effect of isoprenaline before (●) and after (○) addition of propranolol 10^{-7} M to the perfusion medium. Inset, response to isoprenaline (Iso) 10^{-6} M in a control tissue. Responses are expressed as a percentage of the maximal possible relaxation (i.e., complete reversal of the tension developed by the tissue). Preparations were made to contract with oxygen (Po_2 217–220 mmHg) and were treated with dibenzyline 5×10^{-7} M.

strictor and if formed at extraneural sites, may supplement oxygen action at birth.

The residual contractile response to transmural electrical stimulation after selective blockade of α-adrenoceptors is ascribed to a direct activation of ductal muscle rather than to activation of non-adrenergic, possibly purinergic, nerve fibres. In support of our conclusion is the finding that the optimal stimulus frequency (about 200 Hz) for the dibenzyline-treated ductus, while equalling that reported for the nerve-free umbilical vessels (Somlyo & Somlyo, 1968), far exceeds that reported (<50 Hz) for non-adrenergic, non-cholinergic nerves impinging upon vascular (Burnstock, Crowe & Wong, 1979) and non-vascular (Ambache, Verney & Zar, 1970) smooth muscle. Moreover, the ductus arteriosus, like most blood vessels (Burnstock, 1972), is relaxed by adenosine 5′-triphosphate (ATP) (Kovalčík, 1963).

An interesting aspect of our work was the demonstration of an active β-adrenoceptor mechanism in the guinea-pig ductus. This finding contrasts with the apparent lack of β-adrenoceptors in the main pulmonary artery of the adult guinea-pig (Okpako, 1972) as well as with the relative scarcity of β-adrenoceptors in other adult mammalian arteries (Fleisch, Maling & Brodie, 1970; Day & Dixon, 1971; Fleisch & Hooker, 1976). Because it has been shown that β-adrenoceptor activity decreases with age (Fleisch *et al.*, 1970; Fleisch & Hooker, 1976), the presence of functional receptors in the guinea-pig ductus could simply reflect a general property of all developing vessels. Against this explanation, however, is the fact that β-adrenoceptor function is little developed in the lamb ductus, which points to a special role for β-adrenoceptor-mediated relaxation in the guinea-pig ductus.

From our data, it is impossible to draw conclusions on the role of the adrenergic system in the ductus arteriosus. However, considering that the guinea-pig ductus lacks a prostaglandin-mediated relaxant mechanism (Coceani *et al.*, 1978) it is tempting to speculate that prenatal patency of this vessel depends, at least in part, on the activity of its β-adrenoceptor mechanism. Conversely, activation of α-adrenoceptors may contribute to postnatal closure of the ductus, particularly in the guinea-pig. Consistent with the latter possibility is the finding that in the near term foetus, postganglionic sympathetic fibres increase their discharge during compression of the umbilical cord (Biscoe, Purves & Sampson, 1969) and the suggestion from our work that α-adrenoceptor mechanisms become more effective upon raising the oxygen tension from foetal to adult values.

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