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A COMPARISON *in vitro* OF THE VASOCONSTRICTOR RESPONSES OF THE MESENTERIC ARTERIAL VASCULATURE FROM THE CHICKEN AND THE DUCKLING TO NERVOUS STIMULATION AND TO NORADRENALINE

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1 The vasoconstrictor responses of isolated mesenteric arterial vasculature of 2 to 5 week old domestic chickens (*Gallus domesticus*) and domestic ducklings (*Anas platyrhynchos*) to periarterial nerve stimulation and to intra- and extra-vascular noradrenaline were compared.

2 The tissues were perfused at a constant flow rate (2 ml/min) and the change in perfusion pressure produced by the various stimuli was used as a measure of the vasoconstrictor response. In a further study a constant pressure (50 mmHg)-variable flow system was used to corroborate the findings with the constant flow system.

3 The mean pressure response produced by nervous stimulation in the duckling mesentery (137 ± 62 mmHg) was approximately 3 times greater than that produced in the chicken mesentery (46 ± 29 mmHg; $P < 0.001$). Cocaine hydrochloride (1×10^{-5} M) potentiated the responses in the duckling but not in the chicken.

4 The mean maximum pressure response evoked by intravascular noradrenaline in the duckling (170 ± 27 mmHg) was significantly greater than that in the chicken (92 ± 32 mmHg; $P < 0.001$). Cocaine produced a similar degree of potentiation in the 2 species.

5 The mean maximum pressure response evoked by extravascular noradrenaline in the chicken (70 ± 23 mmHg) was significantly greater than that in the duckling (36 ± 25 mmHg; $P < 0.001$) which was the converse of the effect for intravascular noradrenaline. Cocaine produced a much greater potentiation of the responses to extravascular noradrenaline in the duckling than in the chicken.

6 The results from the constant pressure study were similar to the corresponding findings in the constant flow studies. Nervous stimulation arrested flow in the duckling mesentery but not in the chicken. The maximum reduction in flow rate produced by intravascular noradrenaline was significantly greater in the duckling than in the chicken ($P < 0.001$).

7 Quantitative histological studies were performed on transverse sections of arteries prepared with haematoxylin and eosin staining and histochemical fluorescence from 4 chickens and 4 ducklings. The mean wall thickness:lumen diameter ratios of the primary and secondary branches of the duckling mesenteric arterial vasculature were 1.8 and 4.3 times greater than those of the chicken respectively ($P < 0.05$ and $P < 0.001$). The mean density of noradrenergic innervation of the main artery and its primary branches in the duckling was 1.7 and 2.4 times greater than that of the chicken respectively ($P < 0.05$ and $P < 0.01$).

8 The functional differences demonstrated in this study can be explained, at least partially, on the basis of the structural differences observed. During diving in the duck, intense peripheral vasoconstriction is believed to conserve the limited oxygen stores for those tissues most sensitive to oxygen lack. The structural and functional findings in the present study reveal that the duckling mesenteric arterial vasculature is well adapted to produce powerful vasoconstriction and hence play its rôle in oxygen conservation during diving.

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Introduction

It is well documented that ducks can survive asphyxia under water for approximately five times as long as hens (Irving, 1939; Andersen, 1966). During diving in the duck intense vasoconstriction occurs in peripheral vascular beds including the mesentery (but excluding the heart and brain), which markedly reduces or abolishes blood flow to a large portion of the animals's body (Hollenberg & Uvnas, 1963; Johansen, 1964; Djojosugito, Folkow & Yonce, 1969). This mechanism has been shown to conserve the limited oxygen stores in the duck for those tissues that are most sensitive to oxygen lack, in particular the heart and the brain (Butler & Jones, 1971). It might therefore be suspected that the peripheral arterial vasculature in the duck possesses a more sensitive and efficient vasoconstrictor mechanism than the corresponding vessels in the hen.

The primary objective of the present study was to compare the vasoconstrictor responses of the mesenteric arterial vasculature from the chicken and the duckling to nervous stimulation and to intra- and extra-vascular noradrenaline. In parallel with this study the histological structure of arteries in this vascular bed in the two species was examined. An attempt was made to relate the functional findings with the structural observations. Some of the findings have been reported briefly elsewhere (Gooden, 1978a).

Methods

The animals used in this study were 2 to 5 week old domestic chickens (*Gallus domesticus*) and domestic ducklings (*Anas platyrhynchos*). The breed of chicken was the Rhode Island Red-Light Sussex cross and the breed of duckling was the Khaki Campbell. The diving response of the Khaki Campbell has been studied closely by Jones (1973). The animals were anaesthetized with ether and killed by opening the thorax. The anterior mesenteric artery was isolated and cannulated. The mesentery was cut carefully away at its border with the gut using the method described by McGregor (1965; 1971) for the rat and chicken isolated mesentery.

Constant flow rate experiments

The constant flow rate system is a well documented method of examining the vasoconstrictor responses of isolated vascular beds and single blood vessels (Fastier & Smirk, 1947; da la Lande & Rand, 1965). This technique has been used to examine the responses of isolated arterial vasculature in several species including the rat and the chicken (McGregor, 1965; 1971). The apparatus used in the present study was based on that described by de la Lande & Harvey (1965). The perfusate used was that described by Bolton & Bowman (1969) for use with vascular smooth muscle of domestic fowl. A roller pump (LKB Varioperpex type 1200) pumped the perfusate at a rate of 2 ml/min through the perfusion line to the tissue mounted in an organ bath. A heating coil warmed the perfusate to 41°C and the liquid in the organ bath was maintained at the same temperature by a heating jacket. The perfusate in the reservoir and the bath liquid were bubbled with 95% O_2 and 5% CO_2.

To examine the responses of the preparations to nervous stimulation, periarterial nerve stimulation was produced by two platinum ring electrodes around the main artery. This position facilitated periarterial nerve stimulation as described by previous workers (de la Lande & Rand, 1965; McGregor, 1965). The electrical stimulation was applied usually over a logarithmic range of frequencies using supramaximal voltage, a pulse width of 0.2 ms and durations of stimulation specified below. To provide supportive evidence that the electrical stimulation was producing its effect on the vascular bed via the nerve supply, tetrodotoxin, a specific reversible neurotoxin, was added to the organ bath to produce a concentration of 2×10^{-6} M. The presence of this neurotoxin abolished the response to electrical stimulation in both species. Repeated washing of the bath with fresh perfusate resulted in recovery of the pre-tetrodotoxin response. In addition, the particular parameters of electrical stimulation used in these studies were the same or similar to those used by other workers to stimulate chicken blood vessels via periarterial nerves (Bell, 1969; Bennett & Malmfors, 1970). Pulse widths of less than 1.0 ms are generally considered to stimulate nerves and not smooth muscle (e.g. Paton & Vane, 1963).

To examine the responses to intravascular noradrenaline, (−)-noradrenaline bitartrate was injected in 0.1 ml volumes into the perfusion line via rubber tubing adjacent to the tissue. The responses to extravascular noradrenaline were studied by adding noradrenaline to the organ bath.

Changes in perfusion pressure produced by either nerve stimulation or noradrenaline were detected by a pressure transducer (Bell & Howell) and recorded on a potentiometric flat bed recorder (Servoscribe). Mean baseline perfusion pressure for the chicken preparations was 52 ± 12 mmHg (mean ± s.d.; $n = 21$) and for the duckling preparations 49 ± 11 mmHg ($n = 24$).

Nervous stimulation experiments

Constant stimulus train duration over a range of frequencies Preparations were stimulated at frequencies

ranging logarithmically usually from 1.25 to 80 Hz, for a duration of 28 s. This stimulus train duration had been shown by preliminary experiments to give optimum responses for repeated stimulation over a period of 2 to 3 h. In 8 chicken and 8 duckling experiments especially low frequencies of 0.31 and 0.62 Hz were employed.

To investigate the effect of cocaine on the log frequency-response curves, some tissues were perfused for the remainder of the experiment with perfusate containing 1×10^{-5} M cocaine hydrochloride. This concentration has been shown to produce 95% inhibition of noradrenaline uptake$_1$ in the isolated rat heart (Iversen, 1967). A 20 min rest period allowed the tissues to equilibrate with the solution containing cocaine and then the log frequency-response curves were re-established.

Different stimulus train lengths over a range of frequencies At each frequency, ranging logarithmically from 1.25 to 80 Hz, preparations were stimulated with stimulus train lengths ranging from 4 to 1024 impulses. A pulse gating unit was connected between the stimulator and the stimulating electrodes in order to deliver the desired stimulus train length.

Noradrenaline experiments

Intravascular noradrenaline Log dose-response curves were generated by injecting 0.1 ml volumes of noradrenaline into the perfusion line over a logarithmic range of concentration from 1×10^{-6} M to 8×10^{-3} M. To examine the effect of cocaine on the log dose-response curves, some tissues were perfused for the remainder of the experiment with perfusate containing 1×10^{-5} M cocaine hydrochloride. After a 20 min equilibration period the log dose-response curves were re-established.

Extravascular noradrenaline Log concentration-response curves were generated by producing a logarithmic range of organ bath concentrations of noradrenaline from 1×10^{-6} to 4×10^{-3} M. To investigate the effect of cocaine on the log concentration-response curves, some tissues were perfused for the remainder of the experiment with perfusate containing 1×10^{-5} M cocaine hydrochloride. After a 20 min equilibration period the log concentration-response curves were re-established.

Constant pressure experiments

Although the constant flow system is frequently used to examine vasoconstrictor responses of isolated vasculature, it may be a poor representation of the *in vivo* situation. Possibly a more realistic model would be a system in which pressure is held constant and flow is free to vary. Two studies were performed in which a constant pressure system was used in order to corroborate the findings with the constant flow system.

A constant pressure system was produced by arranging the perfusion apparatus vertically, thereby providing a constant hydrostatic pressure head at the level of the organ bath of 50 mmHg. This technique was based on the apparatus described by Fastier & Smirk (1943). The perfusion pressure was chosen to be approximately the same as the resting perfusion pressure in the constant flow studies in both species. Flow through the system was measured in drops/min by a photoelectric drop counter. In other respects the apparatus was basically the same as that used in the constant flow studies.

Nervous stimulation experiments

Constant stimulus train duration over a range of frequencies This procedure was exactly the same as that described for the constant flow experiments.

Intravascular noradrenaline experiments This procedure was exactly as described for the constant flow experiments but did not include the application of cocaine.

Histological studies

The main anterior mesenteric artery, its primary and secondary branches from 4 chickens and 4 ducklings were stained with haematoxylin and eosin. Measurement of the cross sectional vessel dimensions on 6 to 12 sections of each artery sampled provided the data for the calculation of wall thickness to lumen diameter ratios. In order to quantitate the density of noradrenergic innervation of the arteries, samples were prepared from 4 chickens and 4 ducklings using the histochemical fluorescence method of Falck & Owman (1965). The number of fluorescent spots was counted individually from enlarged images of cross sections projected onto a ground glass screen. The density of innervation was expressed as the number of fluorescent spots per mm^2 of adventitia.

Statistical analysis

The responses to nervous stimulation or noradrenaline during the constant flow study were expressed as the change in perfusion pressure from the prestimulus baseline. The responses to these stimuli during the constant pressure study were expressed as a percentage of the flow rate immediately preceding the particular procedure. The comparison of absolute data was performed by the application of two-tailed *t* tests, either paired or unpaired depending upon the

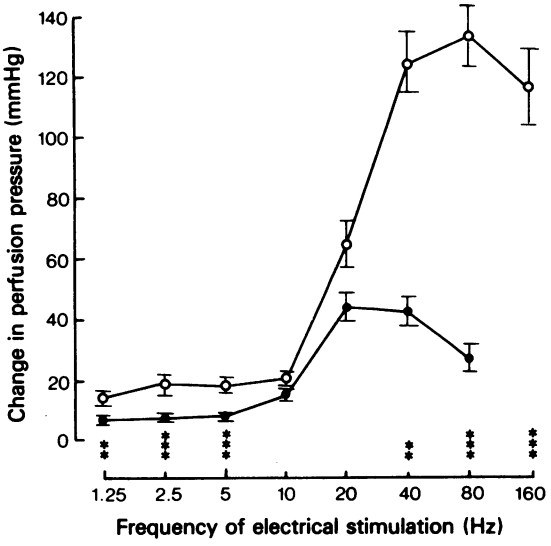

Figure 1 Log frequency-response graphs for periarterially stimulated perfused mesentery from the chicken (●, $n = 39$) and the duckling (○, $n = 35$). The standard error of the mean is shown as a vertical bar. Abscissa scale: frequency of stimulation in Hz on a log scale. Ordinate scale: constrictor response expressed as the increase in perfusion pressure in mmHg. A significant difference between chicken and duckling is shown thus: ** $P < 0.01$; *** $P < 0.001$.

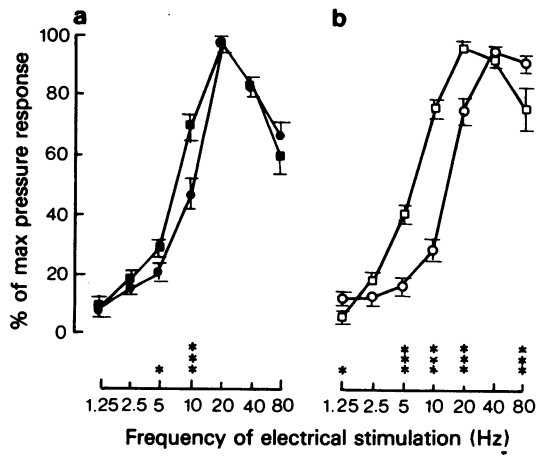

Figure 2 Log frequency-response graphs for periarterially stimulated perfused mesentery from (a) the chicken (closed symbols, $n = 28$) and (b) the duckling (open symbols, $n = 22$) without (circles) and with (squares) cocaine (1×10^{-5} M) in the perfusate and bath liquid. The standard error of the mean is shown as a vertical bar. Abscissa scale: frequency of stimulation in Hz on a log scale. Ordinate scale: constrictor response expressed as a percentage of the maximum response. A significant difference between chicken and duckling is shown thus: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

particular comparison. Where the data were expressed as percentages, the arcsin transformation was used to normalise the distribution of data (Bishop, 1966). A P value less than 0.05 was considered to be statistically significant.

Results

Constant flow rate experiments

Responses to nervous stimulation at a constant stimulus train duration over a range of frequencies The average absolute change in perfusion pressure produced by nervous stimulation for 28 s was significantly greater in the duckling over the range of comparable frequencies except 10 Hz (Figure 1). The maximum response generated in the chicken vasculature regardless of frequency was 46 ± 29 mmHg (mean ± s.d.) and in the duckling was 137 ± 62 mmHg. The difference between the two species was highly significant ($P < 0.001$).

Effect of cocaine on the frequency-response curve Cocaine in the duckling preparations produced a well defined shift of the frequency-response curve to the left (Figure 2). The peak response which occurred at 40 Hz before cocaine, was produced by 20 Hz with cocaine. In general, equivalent responses could be produced after cocaine by half of the pre-cocaine frequency. In the chicken preparations cocaine did not produce such a shift. The frequency at which the maximum response occurred remained at 20 Hz.

Response to nervous stimulation at different stimulus train lengths over a range of frequencies At lower frequencies of stimulation, namely 1.25 to 5 Hz, the duckling tissues gave significantly greater responses than those of the chicken for shorter stimulus train lengths from 4 to 64 pulses. However, at higher frequencies of 20 to 80 Hz, duckling mesenteries produced significantly greater responses at the longer stimulus train lengths of 256 to 1024 pulses. These findings suggested that the duration of stimulation, that is, pulse train length/frequency, might influence the relative responses to the two species. An examination of these data revealed that a duration of stimulation of 13 s produced a significantly greater response in the duckling tissues over the entire range of frequencies tested. Similarly a duration of 6.4 s

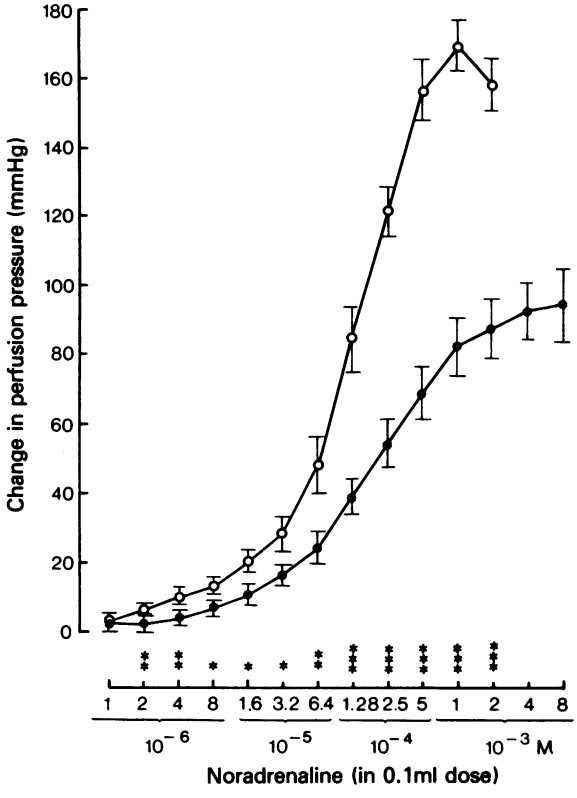

Figure 3 Log dose-response graphs to intravascular noradrenaline for the perfused mesentery from the chicken (●, $n = 16$) and the duckling (○, $n = 13$). The standard error of the mean is shown as a vertical bar. Abscissa scale: molar concentration of noradrenaline, on a log scale, of the 0.1 ml injections administered via the perfusion line. Ordinate scale: constrictor response expressed as the increase in perfusion pressure in mmHg. A significant difference between chicken and duckling is shown thus: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

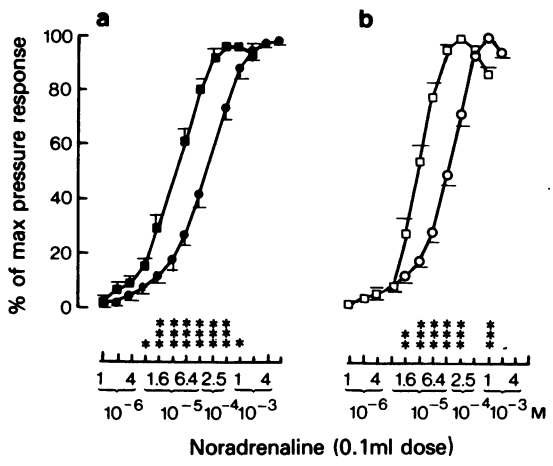

Figure 4 Log dose-response graphs to intravascular noradrenaline for the perfused mesentery from (a) the chicken (closed symbols, $n = 16$) and (b) the duckling (open symbols, $n = 13$) without (circles) and with (squares) cocaine (1×10^{-5} M) in the perfusate and bath liquid. The standard error of the mean is shown as a vertical bar. Abscissa scale: molar concentration of noradrenaline, on a log scale, of the 0.1 ml injections administered via the perfusion line. Ordinate scale: constrictor response expressed as a percentage of the maximum response. A significant difference between chicken and duckling is shown thus: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

evoked a significantly greater response in the duckling at 6 of the 7 frequencies used and a duration of 26 s at 5 of 6 frequencies tested.

Responses to intravascular noradrenaline The average absolute change in perfusion pressure produced by intravascular noradrenaline was significantly greater in the duckling preparations for all of the comparable concentrations of noradrenaline except at the lowest concentration, namely 1×10^{-6} M (Figure 3). The mean maximum pressure response generated by the chicken vasculature, regardless of the concentration of noradrenaline, was 92 ± 32 mmHg and by the duckling tissues 170 ± 27 mmHg. The difference between these responses from the 2 species was highly significant ($P < 0.001$).

Effect of cocaine on the responses to intravascular noradrenaline Cocaine clearly shifted to the left the dose-response curves produced by intravascular noradrenaline in both the chicken and the duckling (Figure 4). However, there was little difference between the degree of this shift in the two species (Table 1A).

Responses to extravascular noradrenaline The average absolute change in perfusion pressure produced by extravascular noradrenaline was significantly greater in the chicken for all the comparable concentrations of noradrenaline except at the lowest concentration, namely 1×10^{-6} M (Figure 5). The mean maximum pressure change generated by the chicken vasculature irrespective of noradrenaline concentration was 70 ± 23 mmHg and by the duckling 36 ± 25 mmHg. The difference between the 2 species was highly significant ($P < 0.001$).

Effect of cocaine on the responses to extravascular noradrenaline Cocaine shifted to the left the concentration-response curves produced by extravascular noradrenaline in both species (Figure 6). However, the degree of this shift was much more pronounced in the duckling. A marked potentiation of responses to noradrenaline concentrations from 4×10^{-6} to 6.4×10^{-5} M was observed in the duckling (Table 1B).

Constant perfusion pressure experiments

Responses to nervous stimulation at a constant stimulus train duration over a range of frequencies Mean resting flow rate in the chicken tissues was 34 drops/min and in the duckling 38 drops/min. This baseline flow rate was equivalent to 1.9 ± 0.4 ml/min in the chicken preparations ($n = 10$) and 2.1 ± 0.3 ml/min in the duckling preparations ($n = 8$). Because of this difference in flow rate, the change in flow produced during stimulation was expressed as a percentage of the control flow rate. Nervous stimulation produced a significantly greater percentage reduction in flow rate in the duckling over the whole range of comparable frequencies (Figure 7). The duckling vasculature was so sensitive that even when especially low frequencies less than 1.25 Hz were used, flow was still reduced. In the chicken the mean minimum flow rate produced regardless of frequency was 38 ± 17% (mean ± s.d.) of the control value and in the duckling 2 ± 6%. The difference between the two species was highly significant ($P < 0.001$). At 20 Hz flow was actually arrested in 4 out of 8 duckling preparations and at 40 Hz flow was arrested in 7 of the 8. Flow was not arrested at any frequency in any of the chicken preparations.

Responses to intravascular noradrenaline Intravascular noradrenaline produced a significantly greater percentage reduction in flow rate in the duckling over the whole range of comparable concentrations of noradrenaline (Figure 8). In the chicken the mean minimum flow rate produced, regardless of noradrenaline concentration, was 23 ± 22% of the control level in the chicken and 1 ± 1% in the duckling ($P < 0.01$). At an injected concentration of 5×10^{-4} M, for example, the minimum flow rate in the chicken was 40 ± 19% but in the duckling was 2 ± 1%.

Histology

Wall thickness: lumen diameter ratios There was no significant difference in the overall diameters of the small mesenteric arteries from the duckling and chicken vasculature. The mean diameter of the primary branches from the main anterior mesenteric artery was of the order of 250 μm and of the secondary branches 200 μm. (Details of structural dimensions of the mesenteric arteries in the 2 species have

Table 1 The degree of potentiation produced by cocaine in the response to (A) intravascular and (B) extravascular noradrenaline expressed as a ratio of (the response with cocaine):(the response without cocaine)

Noradrenaline (M)	*Degree of potentiation* (*Response with cocaine*)/(*Response without cocaine*) *Chicken*	*Duckling*
	(A) *Intravascular noradrenaline*	
	($n = 16$)	($n = 13$)
1.6×10^{-5}	2.3	2.3
3.2×10^{-5}	2.4	3.2
6.4×10^{-5}	2.3	2.8
1.28×10^{-4}	1.9	1.9
2.5×10^{-4}	1.6	1.4
	(B) *Extravascular noradrenaline*	
	($n = 12$)	($n = 10$)
4×10^{-6}	4.5	11.2
8×10^{-6}	5.3	18.3
1.6×10^{-5}	3.7	19.8
3.2×10^{-5}	2.6	10.5
6.4×10^{-5}	1.9	6.1
1.28×10^{-4}	1.6	2.5

The table includes only the noradrenaline concentrations at which there was a significant difference between the percentage responses with and without cocaine (see Figures 4 and 6).

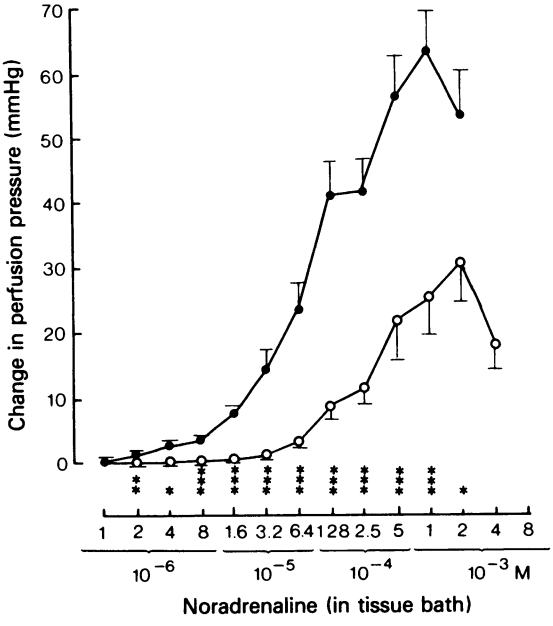

Figure 5 Log concentration-response graphs to extravascular noradrenaline for the perfused mesentery from the chicken (●, $n = 22$) and the duckling (○, $n = 20$). The standard error of the mean shown as a vertical bar. Abscissa scale: molar concentration of noradrenaline, on a log scale, in the bath liquid. Ordinate scale. constrictor response expressed as the increase in perfusion pressure in mmHg. A significant difference between chicken and duckling is shown thus: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

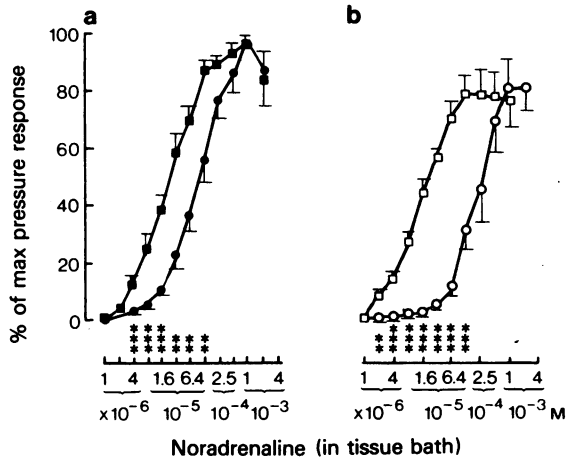

Figure 6 Log concentration-response graphs to extravascular noradrenaline for the perfused mesentery from (a) the chicken (closed symbols, $n = 12$) and (b) the duckling (open symbols, $n = 10$) without (circles) and with (squares) cocaine (1×10^{-5} M) in the perfusate and the bath liquid. The standard error of the mean is shown as a vertical bar. Abscissa scale: molar concentration of noradrenaline, on a log scale, in the bath liquid. Ordinate scale: constrictor response expressed as a percentage of the maximum response. A significant difference between chicken and duckling is shown thus: ** $P < 0.01$; *** $P < 0.001$.

been given elsewhere; Gooden, 1978b). The ratio of wall thickness:lumen diameter was significantly greater in the duckling in both primary ($P < 0.05$) and secondary branches ($P < 0.001$) (Figure 9). This difference in ratio resulted from a combination of a greater wall thickness and a smaller lumen diameter in the duckling arteries. The main artery showed no significant difference in this ratio between the 2 species.

Density of innervation In the main anterior mesenteric artery the fluorescent spots were most densely arranged in the connective tissue coat between the inner circular muscle and the outer longitudinal muscle coats. However, spots were also scattered throughout the longitudinal muscle coat.

When the number of fluorescent spots was related to the area of adventitia containing them, the density of innervation was found to be significantly greater in the duckling main artery ($P < 0.05$) and primary branches ($P < 0.01$) when compared with similar vessels from the chicken (Figure 10). Quantitative measurements were not made in the secondary branches but the density of innervation appeared to be qualitatively similar to that of the primary branches.

Discussion

Both the constant flow and the constant pressure studies demonstrated that the duckling mesenteric arterial vasculature was capable of generating a more marked vasoconstrictor response to both nervous stimulation and intravascular noradrenaline. The maximum pressure response evoked by nervous stimulation in the duckling was approximately 3 times as great as that produced by the chicken. The frequency-response curve of the chicken vasculature was similar to that of mammalian tissue (Girling, 1952; Folkow, 1952; Celander & Folkow, 1953; McGregor, 1965). On the other hand the duckling

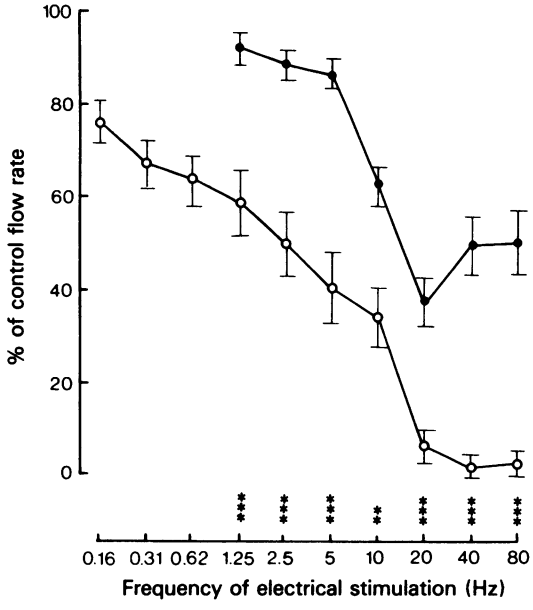

Figure 7 Log frequency-response graphs for periarterially stimulated perfused mesentery from the chicken (●, $n = 10$) and the duckling (○, $n = 8$). The standard error of the mean is shown as a vertical bar. Abscissa scale: frequency of stimulation in Hz on a log scale. Ordinate scale: the lowest flow rate during the period of stimulation expressed as a percentage of the control flow rate. A significant difference between chicken and duckling is shown thus: ** $P < 0.01$; *** $P < 0.001$.

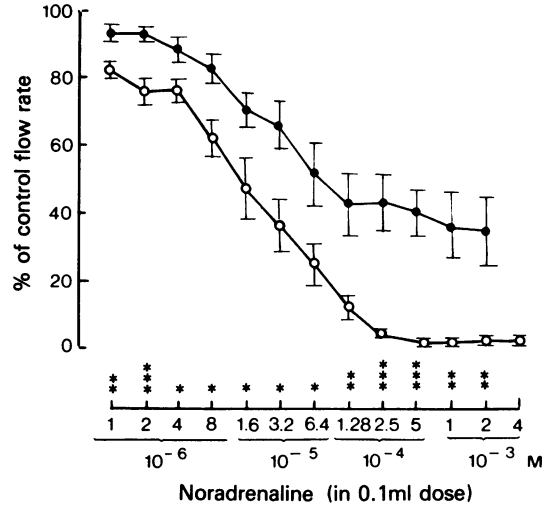

Figure 8 Log dose-response graphs to intravascular noradrenaline for the perfused mesentery from the chicken (●, $n = 9$) and the duckling (○, $n = 8$). The standard error of the mean is shown as a vertical bar. Abscissa scale: molar concentration of noradrenaline on a log scale of 0.1 ml injections via the perfusion line. Ordinate scale: lowest flow rate in response to the noradrenaline expressed as a percentage of the control flow rate. A significant difference between chicken and duckling is shown thus: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

mesenteric bed gave maximum responses at frequencies of nervous stimulation 2 to 4 times higher than those usually reported for mammalian tissue.

The greater pressure response in the duckling reflects the greater increase in total vascular resistance to flow in the mesenteric vascular bed compared with that of the chicken. The vasoconstrictor responses *in vivo* in the vascular bed of the skeletal muscle have been compared in cats and ducks (Folkow, Fuxe & Sonnenschein, 1966). Reflex vasoconstriction was produced in anaesthetized ducks by administration of 20% CO_2 combined with vagotomy and controlled bleeding. The findings in these ducks were compared with similar measurements in anaesthetized cats. However, in the cats vasoconstriction was produced by direct stimulation of the sympathetic nerve supply. The increase in total flow resistance across the skeletal muscle vascular bed was generally far greater in the ducks than in the cats.

There are several possible mechanisms that might be involved in producing the difference in the pressor response between the duckling and the chicken mesenteric arterial beds. The histological investigation in the present study showed that the arterial wall thickness:lumen diameter ratios in the duckling primary and secondary branches were significantly greater than the corresponding values of the chicken vessels. a greater responsiveness of such duckling arteries would be expected on the basis of the theoretical prediction that arteries with a raised wall thickness:lumen diameter ratio will, for a given shortening of their contractile elements, show intensified luminal reductions (Folkow, Grimby & Thulesius, 1958; Folkow & Sivertsson, 1964). In addition the present study showed that the density of noradrenergic innervation of the anterior mesenteric artery and its branches was significantly greater in the duckling compared with the chicken vessels. Folkow *et al.* (1966) reported a qualitatively greater density of noradrenergic innervation in the femoral artery of ducks compared with cats and turkeys. A greater density of innervation would also be expected to potentiate the responsiveness of an arterial bed (Celander & Folkow, 1953). Gillespie & Rae (1972) studied the struc-

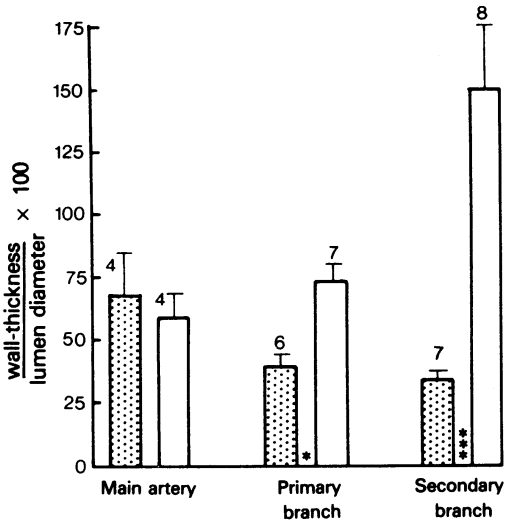

Figure 9 The wall thickness:lumen diameter ratio, expressed as a percentage, in the anterior mesenteric artery and its primary and secondary branches from the chicken (stippled columns, $n = 4$) and the duckling (open columns, $n = 4$). The standard error of the mean is shown as a vertical bar. The number of individual arteries is shown at the top of each column. A significant difference between chicken and duckling is shown thus: * $P < 0.05$; *** $P < 0.001$.

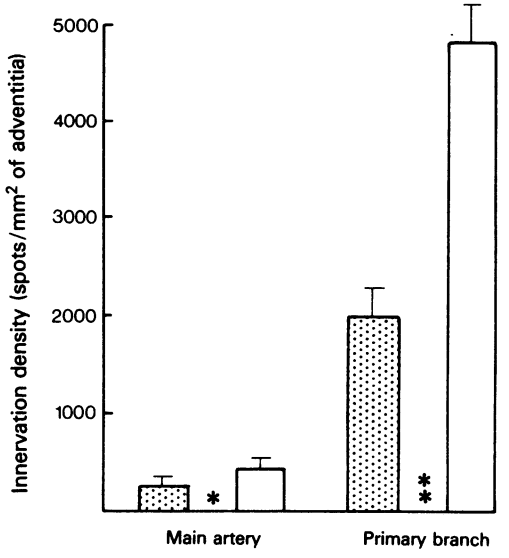

Figure 10 The noradrenergic innervation density expressed as fluorescent spots per mm^2 of adventitia in the anterior mesenteric artery and its primary branches from the chicken (stippled columns, $n = 4$) and the duckling (open columns, $n = 4$). The standard error of the mean is shown as a vertical bar. A significant difference between chicken and duckling is shown thus: * $P < 0.05$; ** < 0.01.

ture and function of the main supply arteries of several vascular beds from rabbit and guinea-pig *in vitro*. The magnitude of the maximum vasoconstrictor response of these arteries to nervous stimulation correlated with the wall thickness:lumen ratio and the density of innervation.

Cocaine produced a shift of the frequency-response curve of the duckling to the left but not that of the chicken. Cocaine is believed to potentiate the action of catecholamines by virtue of its ability to inhibit the uptake of noradrenaline into the nerve terminals (Iversen, 1967). Bevan, Bevan, Purdy, Robinson, Su & Waterson (1972) compared the adrenergic mechanisms of the thoracic aorta and the ear artery of the rabbit, the latter having a greater responsiveness than the former. They compared the histological structure, noradrenaline content and release and uptake of tritiated noradrenaline in these arteries. The percentage reuptake for the thoracic aorta and the ear artery was 29% and 61% respectively. One possible explanation of this difference would be a more efficient uptake process in the nerve terminals of the rabbit ear artery but these workers considered that their findings did not support this theory. A quantitatively denser and wider noradrenergic nerve plexus was present in the ear artery and they suggested that densely packed nerve endings might tend to take up more of the transmitter secreted from nearby nerve terminals. They coined the term 'node crowding' for this effect. This theory could explain the difference in potentiation between the chicken and the duckling with cocaine in the present study but a more efficient uptake process in the duckling nerve terminals is not excluded.

The duckling mesenteric vasculature was generally more responsive to nerve stimulation than the chicken for durations of stimulation from approximately 6 to 30 s regardless of the frequency of stimulation. Dewar (1924) studied the diving habits of wild ducks in detail and observed that the duration of the most frequent dives in male ducks ranged from 14 to 28 s. Thus the range of duration of natural dives of wild ducks is similar to the range of duration of nervous stimulation over which the duckling mesenteric arterial bed gave significantly greater responses than that of the chicken.

The maximum pressure change generated by the duckling mesenteric arterial bed in response to intravascular noradrenaline was significantly greater than that of the chicken for all of the comparable concen-

trations used except the lowest concentration. McGregor (1971), using the same technique as the present study, demonstrated vasoconstrictor responses to intra-arterial noradrenaline in the isolated mesenteric arterial bed of chickens. The difference in the present study in the vasoconstrictor responses between the 2 species may be ascribed at least in part to the greater wall thickness:lumen diameter ratio in the primary and secondary branches of the duckling arterial bed. Differences in the distribution of α- and β-receptors in the two preparations might explain some of the observations in this study but whether such differences exist remains to be elucidated.

A comparison of the maximum pressure response to nervous stimulation with the maximum response to intravascular noradrenaline showed that in both the chicken and the duckling intravascular noradrenaline produced significantly greater responses than nervous stimulation ($P < 0.001$ and $P < 0.01$ respectively). Gillespie & Rae (1972) made similar observations in several arteries from guinea-pig and rabbit. They suggested that nervous stimulation may activate only a relatively small portion of the circular smooth muscle adjacent to the nerve plexus, a concept proposed by Folkow (1964) and supported with experimental evidence by Bevan & Osher (1970).

Both chicken and duckling tissues were significantly less sensitive to noradrenaline applied extravascularly compared with the drug applied intravascularly. De la Lande, Frewin & Waterson (1967) made the same observation in the central ear artery of the rabbit. Their findings indicated that noradrenaline applied extravascularly underwent considerable loss by uptake into the storage sites before it reached the underlying smooth muscle. The maximum pressure response generated by extravascular noradrenaline was significantly greater in the chicken compared with the duckling. Cocaine potentiated the response to extravascular noradrenaline to a much greater extent in the duckling compared with the chicken but potentiated the response to intravascular noradrenaline to approximately the same extent in both species. These findings probably reflect the greater density of noradrenergic innervation of the duckling arteries and hence the greater potential for uptake of noradrenaline.

Using the constant pressure system, flow could be arrested by nervous stimulation in the duckling but not in the chicken. The ability of the duckling vascular bed greatly to reduce or arrest flow in this *in vitro* study is compatible with previous *in vivo* work (Hollenberg & Uvnas, 1963; Johansen, 1964). Intravascular noradrenaline produced significantly greater maximum reduction in flow in the duckling compared with the chicken. Dresel & Wallentin (1966) examined changes in intestinal vascular resistance *in vivo* in the cat and found that the responses to stimulating vasoconstrictor fibres and to infusing noradrenaline were essentially the same whether the pressure or flow was kept constant.

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