Evans Blue Dye and the Development of "Myogenic Tone" in Perfused Resistance Vessels[1] (35716)

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Previous studies in this laboratory have shown that isolated resistance vessels (80 to 350 μ o.d.) from rat skeletal muscle, but not from mesentery, develop myogenic tone when perfused with physiological salt solution (PSS) (1, 2). Further investigation of this phenomenon (3) has raised concern about the extent to which the dye (Evans blue) used to facilitate the dissection procedure contributes to the development of tone. The present study extends these observations concerning the effect of the dye and suggests an explanation of the mechanism of its action.

Methods. Vessels for this study were from 24 male Sprague-Dawley rats, 220 to 480 g in weight. The basic method of dissection and perfusion has been described previously (4). Briefly, a rat was killed by a blow on the head; and a small arterial arborization was isolated from mesentery or skeletal muscle. This vessel segment (220 to 400 μ o.d. and 2–4 mm long) was prepared for perfusion by tying off all of its branches except the one to be studied. The proximal end of the arborization was cannulated with a 22 gauge needle and perfusion was at constant flow rate. Perfusion pressure was monitored via a side arm of the cannula. At the beginning of an experiment, the flow rate was adjusted to give a perfusion pressure of approximately 30 mm Hg. At this perfusion pressure, flow rate was approximately 1 ml/min. The composition of the physiological salt solution (PSS) (moles/liter) was: NaCl, 119; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4 \cdot 7H_2O$, 1.17; $NaHCO_3$, 14.91; dextrose, 5.5; sucrose, 50.0; $CaCl_2$, 16; and CaNa EDTA, 0.026. The perfusion fluid was aerated (95% O_2–5% CO_2) and kept at constant temperature (37°).

Experimental procedures. The current experiments differ from those reported earlier in that the dissection procedure was carried out without the use of dye, except where specified. As a check against the presence of leaks or untied branches, dye was flushed through the segment at the end of each experiment. All injections of $CaCl_2$ were isosmolar and were administered upstream to the pump. Freshly prepared vessels were used in all cases.

Results. Figure 1 shows a tracing obtained from a skeletal muscle artery prepared without the use of Evans blue; no tone (*i.e.*, elevation in pressure above initial or base line value) was apparent in this vessel during 90 min of perfusion. This is in contrast with earlier experiments with arterial segments of comparable size, but exposed to Evans blue during dissection (1, 2), in which tone developed after 20 to 30 min of perfusion. However, when the dye (Evans blue, 3 mg in 3 ml of PSS) was perfused through the vessel segment, tone developed within a few minutes and remained at a high level despite repeated rinsing. When the perfusion fluid was replaced by a Ca-free solution, tone decreased to the initial level, showing that the elevation in perfusion pressure was due to active contraction of the vascular smooth muscle and not to an artifact of perfusion with the dye. Tone returned when perfusion with regular PSS was reinstituted, demonstrating that the change produced by Evans blue was not reversible. Other skeletal muscle segments have been for periods up to 5 hr, in

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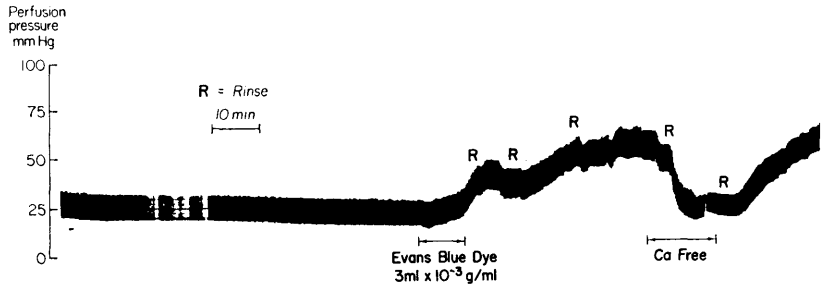

FIG. 1. Effect of Evans blue dye on perfusion pressure of isolated skeletal muscle artery: No dye was used during the dissection. No tone develops during 90 min of perfusion. Tone appears within minutes after the dye is added to the perfusate and persists despite repeated rinsing with dye-free solution. Perfusion with Ca-free PSS abolishes tone Tone returns when perfusion with 1.6 m*M* Ca PSS is reinstated.

the absence of Evans blue without developing tone.

The effect of Evans blue on development of tone in skeletal and mesenteric arteries is summarized in Fig. 2. The control periods without dye ranged from 90 to 480 min. In the case of skeletal muscle arteries (left panel) only two segments showed a measurable elevation in perfusion pressure during the control period. However, after the injection of Evans blue there was a marked elevation in perfusion pressure in all the skeletal muscle vessels studied. Mesenteric arteries (right panel) also showed no tendency toward the development of tone during the control period, but a moderate increase in tone after injection of the dye.

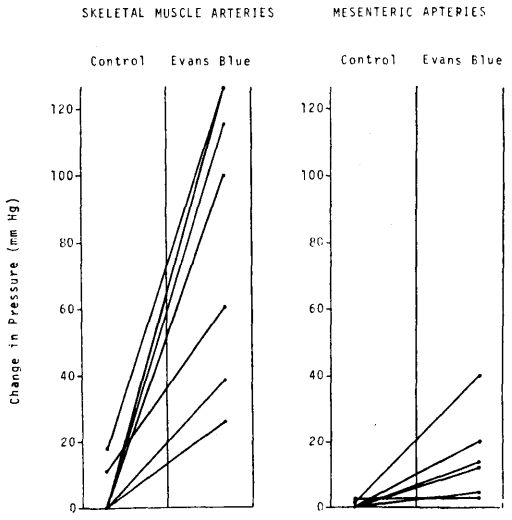

FIG. 2. Effect of Evans blue dye on tone in skeletal and mesenteric arteries: The data are expressed as increase in pressure (mm Hg) above the initial perfusion pressure. The control periods without dye ranged from 90 to 480 min. Tone developed within 3 min after injection of dye.

Adapting the technique described by Waugh (5), the magnitude of pressor responses to slug injections of $CaCl_2$ was used to study the relationship between calcium responsiveness and the induction of tone by Evans blue. Figure 3 shows a tracing from a skeletal muscle artery dissected without dye. After a control period of several hours only a small degree of tone was present, as measured by the fall in perfusion pressure in response to Ca-free perfusion. At this time the vessel exhibited only modest responsiveness to 25-μmole injections of $CaCl_2$. When Evans blue was injected into the perfusate tone developed within a few minutes, and the magnitude of the response to calcium was much enhanced (7 of 7 experiments). Thus, a direct relationship between the induction of tone by the dye and the level of calcium responsiveness is evident. The effect of Evans blue on the development of tone and the potentiation of responsiveness to exogenous boluses of $CaCl_2$ was never as great in mesenteric vessels (6 experiments) as in skeletal muscle.

Discussion. The results of the present study indicate that, when Evans blue dye is not used in the dissection procedure, myogenic tone does not develop in either mesenteric or skeletal muscle arteries. Previously it had been found that, when the dye is used during

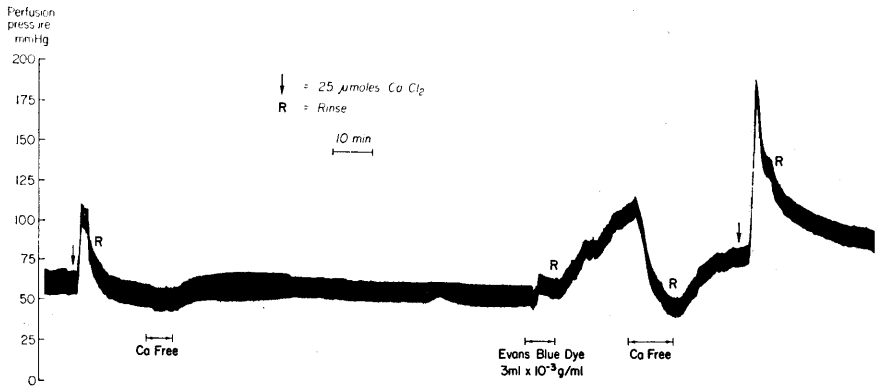

FIG. 3. The effect of Evans blue dye on perfusion pressure and calcium responsiveness of isolated skeletal muscle artery: Prior to injection of dye only a minimal degree of tone is present, as measured by Ca-free perfusion. The response to a 25-μmole bolus of $CaCl_2$ during this control period is small. Shortly after injection of the dye, tone increases and persists despite repeated rinsing. Ca-free perfusion abolishes the tone. Tone returns when perfusion with control PSS is reinstated. At this time the response to the 25 μmole injection of $CaCl_2$ is greatly enhanced.

the dissection, tone does develop in skeletal muscle arteries, but not in mesenteric (1, 2). When the dye is injected into the solution perfusing vessels not previously exposed to the dye, there is a large, persistent increase in perfusion pressure in skeletal muscle segments, and a minor increase in those from mesentery. The effect of the dye on vascular tone is probably not due to alpha receptor stimulation since Uchida and Bohr (1) have shown that the alpha receptor blocker, phentolamine, does not affect tone appreciably.

Since Evans blue is a charged molecule that tends to bind to proteins, it may exert its effect on tone by binding to the proteins of the plasma membrane and increasing its permeability to extracellular calcium. This hypothesis is supported by the observation that Evans blue increases the constrictor response to bolus injections of calcium. Alternatively, the dye may produce its effect by interfering with the extrusion or trapping of calcium.

To postulate any pharmacological significance of the effect of Evans blue on blood vessels *in vivo* is probably unwarranted. From its use in determinations of cardiac output and blood volume, it would appear that the dye has no vasoactive effect *in vivo*. Here it is probably bound to plasma proteins before it has access to the cell membrane. *In vitro*, Evans blue appears to be a useful tool for producing a persistent increase in tonic contraction of vascular smooth muscle.

The observation that Evans blue is much more effective in producing a tonic contraction of the vascular smooth muscle from skeletal muscle than that from the mesentery argues that there is a basic difference between the smooth muscles from these two sites.

Summary. Injection of Evans blue into the perfusate of isolated resistance vessels results in a large, persistent increase in perfusion pressure in skeletal muscle arteries and only a minor increase in mesenteric arteries. The induction of tone is associated with an increase in response to bolus injections of calcium chloride. It is suggested that the dye exerts its effect by increasing cell membrane permeability to extracellular calcium.

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