Simple Isolated Perfused Artery Preparation: Vasoconstrictor Evaluation

CHESTER M. DAVIS and THOMAS F. BURKS ˣ

Abstract □ Small mesenteric arteries free of all extraarterial tissues were obtained from anesthetized dogs and perfused *in vitro* with Krebs solution. Vasoconstrictor responses of these arteries to intraarterial levarterenol and epinephrine were dose related and equivalent to those of arteries surrounded by fat and other tissues. Responsiveness was stable for at least 60 min. This simple preparation is useful for the study of vasoconstrictor phenomena in uncomplicated arterial tissue.

Keyphrases □ Vasoconstrictor evaluation—simple isolated perfused artery preparation described, response to catecholamines determined □ Artery preparation—preparation of simple isolated perfused artery described, vasoconstrictor evaluation □ Vascular smooth muscle—description of simple isolated perfused artery preparation, vasoconstrictor evaluation

It is often desirable in pharmacological analysis to examine drug effects on arterial smooth muscle. Also, an uncomplicated technique for measurement of resistance in small isolated arteries can be advantageous in physiological studies of vascular smooth muscle.

Two methods are generally used in investigations of isolated vascular muscle reactivity: measurement of flow or pressure in perfused vessels, and direct recording of tension or length in vascular muscle strips (1). Smaller vessels that participate in regulation of peripheral resistance have usually not been studied by either method because of difficulty in preparation. The isolated artery preparation described here is relatively simple to prepare and should be easily adaptable to various test procedures.

EXPERIMENTAL

Artery Preparation—Mongrel dogs of either sex, 8–20 kg, were anesthetized with sodium pentobarbital (30 mg/kg) or sodium thiopental (15 mg/kg) and sodium barbital (250 mg/kg) administered intravenously. The anesthetic was supplemented as required during the experiment by administration into a cannulated femoral vein.

The small intestine was exposed by a midline abdominal incision, and an arcade of mesenteric artery supplying a small segment of intestine was chosen for isolation. The mesentery on either side of the arcade was cut with scissors, and umbilical tape ligatures were tied around the intestine on either side of the arterial fan. The mesenteric artery in the arcade chosen was then cannulated with a 4–6-cm length of polyethylene catheter (1.27 mm o.d.; PE 90), usually drawn down to slightly smaller dimensions (1.0 mm o.d.) at its tip and attached to a 10-ml syringe filled with Krebs solution.

After the catheter was tied into the artery, 5 ml of Krebs solution was gently flushed through the vasculature of the gut section to remove any blood. Another ligature was then tied centrally to the cannula, and the artery and periarterial tissue were clipped loose above the cannula and from the serosal surface of the intestine. Each dog was capable of supplying at least 10 such arteries. This procedure provides an "undissected" mesenteric artery, which could be perfused with a physiological salt solution (2).

The mesentery over the artery was then gently split with the tip of an iris forceps along the length of the artery and over the visible branches. With only slight traction on the artery, and with the aid of the iris forceps, the artery was gently dissected free of the periarterial tissues and its small terminals were cut wherever desired. It was generally convenient to prepare an artery with terminals of 0.2–0.5 mm o.d. or smaller. One isolated artery is illustrated in Fig. 1. Since the dissection of the artery required no longer than 1 min and small amounts of Krebs solution could be forced through the lumen every few seconds, it was not necessary to moisten the artery with an externally applied saline solution.

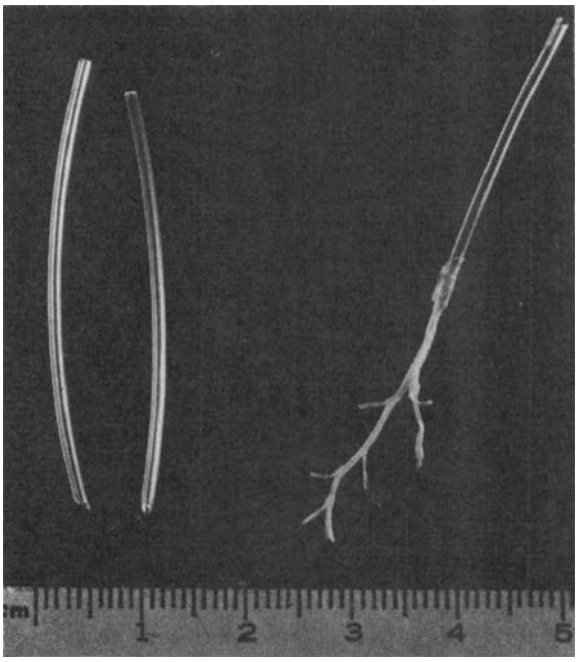

Figure 1—*Photograph of polyethylene cannulas and isolated mesenteric artery. The cannula on the left is the original tubing (PE 90); the one on the right was drawn down to a tip opening of approximately 1 mm o.d. The artery illustrated is typical of the ones employed in this study with terminal vessels less than 0.5 mm in diameter.*

Perfusion—The cannula of the artery was attached to Tygon and polyethylene tubing leading from a constant-flow pulsatile pump[1], and the artery was perfused with Krebs bicarbonate solution warmed to 37° and bubbled with 95% O_2–5% CO_2. The rate of flow varied from 10 to 20 ml/min, but a flow rate of 13 ml/min produced a perfusion pressure in most arteries of 25–30 mm Hg above the resistance offered by the cannula alone. Since flow was held constant, the perfusion pressure was directly proportional to the arterial resistance. Perfusion pressure was measured from a T-connection between the pump and the artery by a pressure transducer[2] and was recorded on an oscillographic recorder[3].

Intraarterial injections were made with a 100-μl syringe in volumes of 0.01–0.1 ml into injectable tubing immediately above the arterial cannula. A 2–3-cm section of injectable tubing was prepared by wrapping one or two thicknesses of adhesive tape around ordinary rubber tubing.

The artery was usually suspended from the cannula into the

[1] Sigmamotor type T-8.
[2] Statham P23Db.
[3] Beckman type RM dynograph.

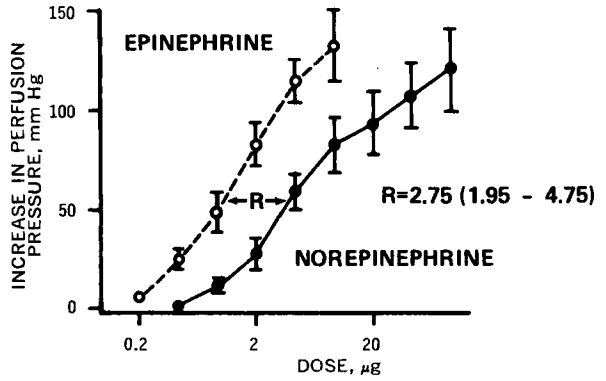

Figure 2—*Dose–response relationships of pressor responses (changes in perfusion pressure) to epinephrine and levarterenol (norepinephrine) administered intraarterially in isolated mesenteric arteries. R indicates that 1 dose unit of epinephrine produced an increase in perfusion pressure equivalent to 2.75 units of levarterenol. Each point is the mean (±SEM) of responses in preparations from six animals.*

warm, moist air of the muscle chamber of a conventional isolated muscle bath, and the perfusate was allowed to drain from the bottom of the bath. In some experiments, the artery was exposed to room air without observable changes in responsiveness.

Drugs and Chemicals—The vasoconstrictor agents employed were levarterenol (norepinephrine) bitartrate[4] and epinephrine bitartrate[5]. Doses are expressed as the free bases. Krebs bicarbonate solution of the following composition was employed as the perfusion medium (in millimoles): NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.1; $MgSO_4$, 0.5; $NaHCO_3$, 25.0; and glucose, 10.0.

Experimental Design—Two groups of six dogs were used. In one group, two dissected arteries were removed from each dog and each artery was injected with all dose levels of either levarterenol or epinephrine. These responses were employed in construction of dose–response curves. In the other groups of animals, six undissected arteries were removed from each dog and were attached to the perfusion apparatus. After their responsiveness to levarterenol and epinephrine stabilized, extraarterial tissues were removed and responses of the dissected arteries to the adrenergic amines were determined.

Effects of removing extraarterial tissues on responsiveness were compared by Student's *t* test, paired comparisons (3). Dose–response curves were compared by parallel line bioassay (4). Relative differences in arterial reactivity among dogs and by position removed (whether first or sixth) were determined by a Latin-square design analysis of variance (5).

RESULTS

The arteries were removed quickly and without difficulty from the donor animals. Once perfusion was begun, the baseline perfusion pressure usually stabilized within 5–10 min and remained relatively constant for at least 60 min. Test doses of catecholamine were administered at 2-min intervals beginning about 5 min after perfusion was begun. Vasoconstrictor responses to the intraarterial catecholamines became maximal within 10–20 min and remained stable for 30–60 min.

A uniform time interval (1.5–4 min) between doses improved reproducibility of the responses. In most arteries, perfusion pressure after a response returned to baseline within 2 min of the catecholamine injection. Within the limits of 0.01–0.1 ml, the volume of the injection did not affect the magnitude of the responses. There was also no difference in responsiveness whether the solvent for the catecholamines was purified water, 0.9% sodium chloride, or Krebs solution.

There was a significant variation between animals in responsiveness of their isolated arteries to levarterenol and epinephrine. In a series of six dogs, two of the animals provided arteries significantly more reactive than those from the remaining four dogs. Different arteries from the same dog produced comparable responses. The position of the artery removed (whether first or third) did not affect responsiveness to the catecholamines.

[4] Levophed, Winthrop.
[5] Suprarenin, Winthrop.

Table I—Comparative Responsiveness of Isolated Mesenteric Arteries before and after Removal of Extraarterial Tissues (n = 36)

	Before	After	p
Baseline perfusion pressure, mm Hg[a]	26 ± 1	41 ± 3	<0.01
Responses, mm Hg, to:			
Levarterenol (1.6–4.0 μg)	93 ± 4	100 ± 5	NS
Epinephrine (0.8–2.0 μg)	87 ± 5	96 ± 6	NS

[a] Each artery was perfused at identical flow rates before and after clearing extraarterial tissues.

The magnitude of vasoconstrictor responses to intraarterial levarterenol and epinephrine was proportional to the dose levels administered (Fig. 2). Dose–response curves to the two catecholamines were parallel, but epinephrine was 2.7 times more potent than levarterenol as a vasoconstrictor.

To determine whether removal of periarterial tissues from the "undissected" arteries resulted in loss of fine juxtaintestinal arterial branches and whether the imposition of the surgical trauma affected responses, the reactivity of the isolated arteries was compared before and after dissection from periarterial tissue (Table I). Baseline perfusion pressures at identical rates of flow before and after dissection were slightly increased after the dissection procedure. There was no significant change in the magnitude of responses to levarterenol and epinephrine due to dissection. An example of the responses of an artery before and after removal of periarterial tissues is illustrated in Fig. 3.

DISCUSSION

The isolated artery preparation described here provides a measure of arterial resistance, is easily prepared, and is quite reactive to catecholamines. Two further features are reproducibility of responses and absence of fat and other periarterial tissues. The isolated artery preparation introduced by Rogers *et al.* (2) has the advantage of associated periarterial nerves but the disadvantage of a large mass of extraarterial fat and connective tissue which can serve as a reservoir of ions and as a depot for lipid-soluble drugs and hormones. Isolated resistance vessels from the rabbit brain or mesentery, described by Uchida *et al.* (6), provide small (50–250 μm o.d.) terminal vessels but lack the advantage of ease of preparation since a dissection microscope and a small (400 μm o.d.) cannula tip are required.

There was concern that dissection of the artery from surrounding tissues would result in excess surgical trauma or loss of smaller terminal vessels near the intestine. Therefore, responses before and after clearing the arteries of extraarterial tissues were compared. The results showed no decreases in baseline perfusion pressure that would have resulted if fine resistance vessels were removed in the dissection process (6). The pressure changes induced by the catecholamines also were not decreased after dissection.

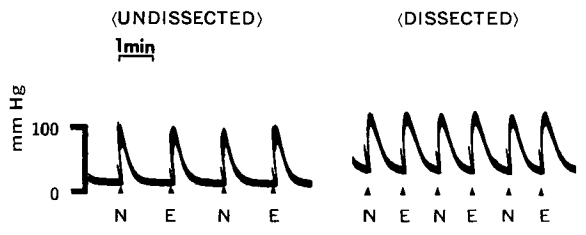

Figure 3—*Responses of an isolated artery to levarterenol (norepinephrine) (N, 3 μg) and to epinephrine (E, 1 μg) before (undissected) and after (dissected) removal of extraarterial fat and other tissues. The artery was perfused at constant flow of 11 ml/min. Baseline perfusion pressure was increased after dissection, but the changes in perfusion pressure produced by the catecholamines were not changed.*

These observations indicate that the major portion of the resistance segments were retained after the dissection process.

Responsiveness to intraarterially injected catecholamines was in the same general range as reported for similar preparations (2, 6). The potency ratio between epinephrine and levarterenol of 2.75 (1.95–4.75) agrees closely with that reported by Rogers *et al.* (2) of 2.61 (1.56–3.66).

This isolated artery preparation can provide a useful method of testing vasoconstrictor responses to catecholamines. The arteries may be perfused while hanging in air or submerged in bath fluid in recirculating or nonrecirculating systems. They also may be stored at least overnight in refrigerated Krebs solution without loss of responsiveness to levarterenol, epinephrine, or potassium ion (7). Therefore, it seems to provide one simple alternative to vascular strips or conduit vessels in studies of vascular smooth muscle.

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N-Substituted Indanamines as Potential Hypoglycemic Agents

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Abstract □ A number of indanamines substituted at the terminal amino nitrogen with various aliphatic, alicyclic, heterocyclic, and aromatic ring systems were synthesized and screened for hypoglycemic activity. None was found to possess significant activity compared to tolbutamide.

Keyphrases □ Indanamines, *N*-substituted—synthesized and screened as hypoglycemic agents □ Hypoglycemic agents, potential—synthesis and screening of *N*-substituted indanamines

Hypoglycemic activity was reported among different types of indanamines (1–4). It was also observed that *n*-butylpyrrolidine alone possesses appreciable hypoglycemic activity but was extremely toxic (1). Furthermore, it is well known that among the sulfonylureas, various alicyclic, heterocyclic, and aromatic ring substitutions at the terminal urea nitrogen atom had a beneficial effect (5–8).

Therefore, attempts were made to incorporate various such ring systems at the terminal amino nitrogen atom of the indanamine ring moiety and to observe the effect on hypoglycemic activity. All of these compounds were prepared by following a reported procedure (1–4). Unlike the previous work, both the amides and the corresponding amine hydrochloride salts were used for the pharmacological evaluation of hypoglycemic activity.

EXPERIMENTAL[1]

Indan-*N*-substituted Carboxamides (Ia and IIa)—These compounds were prepared from indan-1-carboxylic acid (9, 10) (1 mole) and indan-1-acetic acid (11) (1 mole) *via* the acid chloride intermediate (3) by reaction with the corresponding primary or secondary bases (1.5 moles) in the presence of 10% NaOH solution. The amides were either extracted with a suitable solvent or obtained as a crystalline solid, which was subsequently purified by crystallization from suitable solvents. The physical properties, analyses, and hypoglycemic activity of all amides synthesized are given in Table I.

***N*-Substituted Indanamines (I and II)**—The amides (1 mole) so prepared were reduced by lithium aluminum hydride reduction (1.5 moles) in absolute ether. The excess lithium aluminum hydride was decomposed with calculated amounts of 3% NaOH solution and filtered. The filtrate was extracted with cold 2 *N* HCl until it was free from amine. The aqueous layer was basified and extracted with ether, washed, dried, and distilled under reduced pressure.

The amines were characterized as their hydrochloride salts, and these hydrochloride salts were used for hypoglycemic screening. The analyses, physical properties, and hypoglycemic activity of the amines are given in Table I.

[1] All melting points were determined in a Gallenkamp apparatus and are corrected. Boiling points are uncorrected.