A vibrator prevents streaming during close-arterial infusion into the kidney

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Hamza, Shereen M., and Susan Kaufman. A vibrator prevents streaming during close-arterial infusion into the kidney. Am J Physiol Renal Physiol 286: F643–F646, 2004.—Close-arterial infusion of test substances allows one to study the responses of a selected vascular bed without inducing confounding systemic effects. Unfortunately, laminar flow patterns within the artery cause streaming of the injected factor, so that distribution within the target organ is not homogeneous. We describe a reliable method of overcoming these problems. Specifically, we attach a vibrator (i-Vibe egg) to the syringe containing the test substance. We showed that, without vibration, infusion of a solution of Evans blue (0.5% wt/vol) results in uneven distribution of the dye in the kidney. Vibration of the syringe during infusion allows for uniform coloration of the kidney surface. There is also functional improvement of drug distribution during vibration. Renal blood flow was measured during intrarenal infusion of phenoxyphrine (150 μl, 0.05–0.5 μg). Vibration caused a significant leftward shift in the dose-response curve, i.e., the phenoxyphrine-induced reduction in renal blood flow was enhanced by vibration. This cheap, simple method for ensuring adequate mixing of intra-arterially infused substances will facilitate not only the study of renal function in the rat but also infusion of test and therapeutic substances into other organs.

PHARMACOLOGICAL AGENTS are commonly administered during the course of investigating renal function. However, intravenous infusion may cause systemic effects that indirectly influence the kidney. To avoid this problem, the drugs are often infused directly into the renal artery. Unfortunately, laminar flow patterns within arteries cause streaming and uneven distribution of infusate within the organ, with the result that the drug may only partially, if at all, perfuse the vascular beds of interest (4, 18, 20). This can lead to variability of data and potential misinterpretation of the results. The general problem of streaming during intrarterial infusion is also of clinical significance because therapeutic agents, particularly for chemotherapy, may be delivered in this manner to achieve high local concentrations without accompanying systemic toxicity (2, 6, 11–14).

Several investigators described protocols to improve mixing during intra-arterial injection of drugs. These involved introduction of balloon cannulae to cause turbulence (1), pulsed infusion (22), and extracorporeal blood circuits (4). In 1995, Parekh (18) devised a multiple catheter system with a magnetic pump that could draw back blood to premix with the drug to be infused. Although this technique does improve uniform delivery, it is expensive and requires extensive preparation. We describe a simple, inexpensive method to improve drug delivery to the kidney. We showed that this system improves spatial dye distribution, as well as the functional response to phenylephrine.

MATERIALS AND METHODS

The experimental procedures were approved by the local Animal Welfare Committee in accordance with the guidelines issued by the Canada Council on Animal Care. All animals were killed with an anesthetic overdose of pentobarbital sodium at the completion of the each experiment.

Animals and housing. Male Long-Evans rats (350–550 g, Charles River) were housed for at least 1 wk in the University of Alberta Animal Facility. They were maintained on a 0.3% sodium diet and tap water ad libitum in a temperature- and humidity-controlled environment with a 12:12-h light-dark cycle.

Anesthesia and surgery. The animals were injected with pentobarbital sodium (50 mg/kg body wt ip), followed by Inactin [Byck, ethyl-(1-methyl-propyl)-malonyl-thio-urea, 80 mg/kg body wt sc] 1 h later. Body temperature was maintained at 37°C (Homeothermic blanket, Harvard Apparatus).

The left femoral vein was cannulated with Silastic tubing (0.51-mm ID, 0.94-mm OD, Dow Corning) and infused with isotonic saline (3 ml/h). The left femoral artery was cannulated with polyethylene tubing (PE-50 0.58-mm ID, 0.97-mm OD, VWR, Mississauga, Ontario), which was connected to a pressure transducer for online recording of mean arterial pressure (MAP). After a midline laparotomy, the intestines were reflected to the right side of the animal. The stomach was reflected onto the chest and held in position with hemostats. The spleen was detached from the stomach and gently retracted to the left side of the animal. All exteriorized organs were covered with moistened gauze and plastic wrap. The renal artery was carefully separated from the renal vein, ensuring the adventitia was left intact to preserve the renal nerves. A transit-time flow probe (Transonic Systems, Ithaca, NY) was placed around the renal artery distal to its origin from the descending aorta.

Preparation and insertion of renal arterial catheter. A 33-gauge stainless steel needle (Hamilton) was inserted into one end of a 10-cm length of Microline tubing (0.25-mm ID, 0.76-mm OD, Cole-Palmer, Ontario, Canada), which had been stretched to provide a tight seal around the needle. The other end of this tubing was attached to a syringe. With curved forceps, the renal arterial wall was gently held up, while the 33-gauge needle was inserted at the junction of the renal artery and descending aorta (Fig. 1). The needle was advanced ~2 mm into the artery and held in place with a small drop of Vet Bond tissue adhesive (3M Animal Care Products, St. Paul, MN) at its point of entry into the vessel. An immediate flow of blood into the line on insertion indicated patency. It was not necessary at any time to interrupt blood flow to the kidney. The animal was left to stabilize for 40 min while MAP and renal blood flow were monitored.

Experimental protocol. The vibrator (i-Vibe egg, Doc Johnson Enterprises) was attached, with plastic tape (3M Colourflex, Ontario, Canada), to the syringe to be used for dye infusion. Vibration was turned on at the lowest setting ~30 s before the dye was injected. The dye solution (1 ml) was then slowly infused over a second period of 30 s. In the control group, the dye was infused in exactly the same way.

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A similar procedure was followed for the injection of phenylephrine, except that MAP and renal blood flow values were recorded continuously online (DI-151RS, DATAQ Instruments, Adron, OH) using WINDAQ software for analysis (DATAQ Instruments). After stabilization (40 min), baseline measurements were made over 10 min. The phenylephrine was then infused over a period of 30 s at doses of 0, 0.05, 0.15, and 0.5/1000 g dissolved in 150/1000 l isotonic saline. There was a 10-min recovery period between each dose. Each animal was used for both with/without vibration experiments, with the order of testing being alternated between consecutive experiments.

Drugs and solutions. Evans blue (Fisher Scientific, Ontario, Canada) was dissolved in isotonic saline (0.5% wt/vol). Solutions of phenylephrine (Sabex, Quebec, Canada) were made by serial dilution in heparinized isotonic saline (10,000 IU/l).

Analysis of data and statistics. The maximal decrease in renal blood flow following infusion of each dose of phenylephrine was measured. Two-way repeated-measures ANOVA, followed by the Student-Newman-Keuls method for post hoc analysis, was used to determine the statistical significance of the change in renal blood flow between injection with/without vibration. Statistical significance was accepted at P < 0.05. Means ± SE are reported in the figures and text.

RESULTS

Injection of dye directly into the renal artery, with no vibration, resulted in uneven patches of dye accumulation over the surface of the kidney (Fig. 2A). With application of vibration to the syringe during injection, the kidney showed even mottled distribution of color over both dorsal and ventral surfaces (Fig. 2B).

There was no difference between the two groups with respect to baseline renal blood flow (with vibration: 6.3 ± 0.6 ml/min, n = 8; without vibration: 6.5 ± 0.6 ml/min, n = 8). Intra-arterial infusion of the α1-adrenergic agonist phenylephrine induced a dose-dependent reduction in renal blood flow. Vibration caused a leftward shift of the dose-response curve relative to the response in the absence of vibration, i.e., there was a greater decrease in renal blood flow when the phenylephrine injection was accompanied by vibration (Fig. 3). Repeated-measures two-way ANOVA confirmed that there was a significant effect of treatment (with vibration vs. without vi-
VIBRATOR PREVENTS ARTERIAL STREAMING

F645

bration: \( P < 0.001 \). Although systemic blood pressure rose during the phenylephrine infusion, there was never any significant difference between the groups (with/without vibration), even at the highest dose (with vibration: 92.4 ± 2.5 to 101.6 ± 3.2 mmHg, \( n = 8 \); without vibration: 93.2 ± 3.7 to 101 ± 3.3 mmHg, \( n = 8 \)).

DISCUSSION

Direct infusion of Evans blue into the renal artery resulted in uneven delivery of dye to the renal tissue. Application of vibration during the infusion improved dye distribution, so there was uniform coloration on both dorsal and ventral faces of the kidney. There was also functional improvement of drug distribution during vibration. The vascular response to phenylephrine, as reflected by the decrease in renal blood flow, was significantly greater when vibration was applied to the syringe during infusion. We suggest that transmission of vibration, both along the microline tubing and within the fluid column, causes turbulence and mixing as the infusate enters the blood stream. This ensures that as the blood flows through all downstream branches of the renal artery into the kidney, there is homogeneous delivery of a drug to the renal tissue. The greatest effect of vibration on renal blood flow (55% reduction vs. 25% reduction) occurred after infusion of phenylephrine at 1.5 \( \times 10^{-7} \) g (infused over 30 s, at a flow rate of \( \approx 5 \) ml/min). Significantly, this is the concentration of phenylephrine (7.5 \( \times 10^{-7} \) M), which consistently induces vasoconstriction in the isolated, perfused kidney (17, 19).

The issue of streaming in arterial flow has been extensively studied, at least in part, because of the clinical importance of delivering chemotherapeutic drugs to target organs where treatment may be complicated by uneven distribution and focal toxicity (2, 3, 5, 6, 14). Variable delivery of tracer due to streaming has also been demonstrated in life-sized glass models of the human hepatic artery (15), the human carotid artery (11), and the human iliofemoral/pelvic arteries (12), as well as during carotid artery infusion in rats (20). Our data were in agreement with those obtained by Parekh (18) that closearterial infusion of dye into the rat kidney normally results in extremely uneven distribution of coloration.

The cannulation technique and the use of the vibrator offer several advantages over previously reported methods of infusion drugs into the kidney of the rat. In contrast to most other techniques, renal blood flow does not have to be interrupted, even momentarily, during the cannulation procedure. Although Fine et al. (7) acknowledge that blood flow should not be stopped for more than 10 to 20 s, it is our experience that ligating the aorta or renal artery results in immediate almost blanching of the kidney. This will undoubtedly induce both direct (renin release) and indirect (renal afferent nerve activity) responses to alter both renal function and systemic hemodynamics (9, 16).

Another approach has been to cannulate the supraparenchymal artery (10, 21). Not only does this fail to address the problem of streaming and uneven distribution of infusate, but we found that ligation of the supraparenchymal artery induced lability of the MAP, which directly affected renal blood flow (Hamza and Kaufman, unpublished observations).

Cupps and Sonnenberg (4) recognized the need to ensure adequate distribution of an infused drug with blood before entering the kidney. To this end, they used an extracorporeal circuit as devised by Fink and Brody (8). This circuit involved shunting blood from the carotid artery to an aortic pouch leading to the left renal artery. This enabled the test substances to be infused some distance upstream from the kidney, which ensured adequate mixing. The disadvantage of this method lies in the fact that, as admitted by the authors themselves, it is highly invasive.

It was in light of these previous attempts to address the problem of streaming that Parekh (18), in 1995, developed a multiple catheter system with a magnetic pump whereby blood could be drawn back and mixed with the test substance before being reinfused into the animal. Parekh showed convincingly that with this system, not only was injected dye evenly distributed in the kidney, but the renal responses to vasoactive drugs were augmented and the systemic responses were reduced. The disadvantage of this system is that it is complicated to set up, involving as it does focus multiple cannulae and a magnetic membrane pump. By contrast, our method of simply applying steady vibration to the syringe with a commercial vibrator is economical, efficient, and significantly improves drug distribution in the kidney.

We describe applying vibration directly to the infusion syringe. However, the system worked equally well when the vibrator was taped to the hard plastic male adapter on an intravenous infusion set (Abbott Laboratories): this would allow one to use a syringe pump or a peristaltic pump placed upstream of the vibrator to administer the solution. Moreover, one may use either a flank or a midline approach to the kidney, because the vibrator is applied several centimeters distal to the tip of the cannula (Fig. 1). The ability to ensure homogeneous drug distribution during close-arterial infusion is critical to ensuring meaningful, reproducible experimental data, not only in the kidney, but also in other target organs such as the brain and liver.

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