Specific Desensitization of the Canine Renal Vasculature to Angiotensin II Despite Cyclo-Oxygenase Inhibition

LEONARD G. MEGGS, M.D., RICHARD W. KATZBERG, M.D., PETER DeLEEUW, M.D., AND NORMAN K. HOLLENBERG, M.D., Ph.D.

Departments of Medicine and Radiology, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts

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Intrarenal angiotensin (AII) infusion results in a poorly sustained renal vasoconstrictor response. To examine the relationship between fade and renal tachyphylaxis to AII, sub-pressor doses of AII and norepinephrine (NE) were injected into the renal arteries of anesthetized dogs, resulting in a transient reduction (> 50 percent) in renal blood flow. Continuous intrarenal AII infusion, sufficient to reduce renal blood flow by 50 percent, followed. Within five minutes, despite continued AII infusion, substantial recovery (73 ± 11 percent) of renal blood flow occurred; however, the response to AII bolus injection was lost, but that to NE was sustained.

A second group of dogs received indomethacin (5 mg/kg intravenously) 30 minutes prior to the study; the reduction in renal blood flow was better sustained; however, renal tachyphylaxis was still evident.

INTRODUCTION

Despite angiotensin's potent vasoconstrictor properties, a perplexing observation has been its failure to produce a sustained contractile response in vascular smooth muscle [1,2]. Two phenomena, fade and tachyphylaxis, have been implicated. Fade has been described as the diminution of response, despite the continued presence of an agonist [3]. Loss of response may be due to refractoriness or, in the case of vascular smooth muscle, to an alteration in excitation-contraction coupling or to the contractile apparatus itself [4]. In this case the refractory state is generalized, and no agonist would be expected to induce a response. Tachyphylaxis, or specific desensitization, involves specific loss of response to an agonist following repeated or prolonged exposure of an effector system [3]. Loss of contractile response is specific for a single agonist, and the system is capable of responding to alternate agonists.

In this investigation, we have attempted to determine the role of prostaglandins in the poorly sustained renal vascular contractile response to angiotensin and whether prostaglandin-mediated alterations in vascular tone can be dissociated from specific desensitization.

METHODS

Studies were performed in ten mixed-breed dogs of both sexes, weighing 14–28 kg. The animals were kept on ad libitum diet prior to the investigation. Anesthesia was induced with intravenous pentobarbital sodium (30 mg/kg) and maintained with...
additional doses (5 mg/kg) as required. Respiration was controlled with a cuffed endotracheal tube and a respirator pump (Harvard Apparatus).

The left kidney was exposed via a flank incision and an appropriately sized electromagnetic flow probe placed on a proximal segment of the artery. Blood flow was measured either with a Statham flowmeter (Model M 4001) or a Gould Godard flowmeter (Model SP 2202) which were calibrated in vivo. Arterial pressure was measured with a transducer (Statham P 23 D C) from a catheter introduced into the aorta from the femoral artery. Via the contralateral femoral artery a coaxial catheter system was introduced and advanced into the left renal artery under fluoroscopic control; a continuous 0.7 ml/minute infusion of normal saline was maintained with a Harvard infusion pump to prevent clotting of the catheter system. Infusions of angiotensin II (AII) into the renal artery were given through the inner lumen, while bolus injections of AII and norepinephrine (NE) were given through the outer lumen. Pressures and flows were monitored continuously on a pen recorder (Grass Instruments). After blood flow and pressure had stabilized, bolus injections of angiotensin II (Hypertension, Ciba) and norepinephrine (Levophed bitartrate, Winthrop) were injected into the renal artery. For each agent a dose was selected that would produce a fall in renal blood flow of at least 50 percent but would be without effect on systemic pressure. A minimum period of five minutes was allowed between doses for recovery. Ten minutes after the last injection of either agent, one of the two following protocols was performed.

PROTOCOLS

Protocol A

After defining the dose-response for AII and NE in five dogs, a continuous infusion of AII into the renal artery was initiated at a dose (3–5 μg/minute), previously demonstrated to have minimal systemic pressor effects, while reducing renal blood flow by at least 70 percent [5]. After five minutes of continuous AII infusion, bolus injections of AII and NE were repeated. The infusion was discontinued, and bolus injections of AII and NE repeated.

Protocol B

In a second group of five dogs, an identical protocol was followed, but an intravenous injection of the cyclo-oxygenase inhibitor indomethacin (5 mg/kg) was given 30 minutes prior to defining the dose-response curves for AII and NE. Thereafter, a sequence identical to that in Protocol A was followed. The time interval between discontinuing the AII infusion and full recovery of preinfusion bolus AII response was determined for three of five dogs in each protocol. This interval is referred to as recovery period (Fig. 2).

ANALYSIS

The transient reductions in renal blood flow induced by AII and NE were expressed as ΔRBF (ml/minute); ΔRBF was calculated from the baseline renal blood flow, immediately prior to the bolus challenge. Mean values have been presented with the standard error as an index of dispersion. Statistical significance was assessed by either the student t-test for paired data where appropriate, or nonparametric methods such as Wilcoxon's rank sum test (WRST) and Fisher's exact test (FET). The null hypothesis was rejected when a p value of 0.05 or less was attained.
RESULTS

Protocol A

Continuous infusion of $A_{II}$ into the renal artery induced a similar response in each dog. Within 60 seconds renal blood flow decreased from a baseline of $151 \pm 23$ ml/minute to $43 \pm 22$ ml/minute (Fig. 1), a 72 percent reduction ($p < .01$). Thereafter, renal blood flow began to return toward baseline, so that after five minutes of continuous $A_{II}$ infusion, the reduction was $41 \pm 17$ ml/minute or a 27 percent reduction from the baseline renal blood flow. At this time, the vasoconstrictor response to NE was well sustained, but the response to $A_{II}$ was either absent or markedly diminished (Fig. 2).

Protocol B

Pretreatment with indomethacin resulted in a fall in renal blood flow, from $133 \pm 19$ ml/minute to $110 \pm 15$ ml/minute. The $A_{II}$ infusion induced a reduction in renal blood flow resembling that in protocol A qualitatively (Table 1), but differing in the

FIG. 1. Renal vascular responses to $A_{II}$ and NE, prior to (left) and following continuous $A_{II}$ infusion (right). Note that indomethacin pretreatment did not prevent the striking reduction in the responsiveness to $A_{II}$ following the continuous infusion. The reduction in renal blood flow during continuous infusion of $A_{II}$ is better sustained in dogs pretreated with indomethacin.

FIG. 2. Schematic tracing of the renal vascular response to $A_{II}$ and NE, prior to and after the development of tachyphylaxis to $A_{II}$. Following five minutes of continuous $A_{II}$ infusion, the vascular response to bolus $A_{II}$ was lost, while that to NE was sustained. The time interval between discontinuing the $A_{II}$ infusion and full recovery of the bolus $A_{II}$ response is designated the recovery period.
tendency of renal blood flow to return toward baseline. After five minutes of continuous $A_{II}$ infusion, the blood flow reduction was $75 \pm 6$ ml/minute or a 69 percent reduction ($p < .01$).

**DISCUSSION**

The term “tachyphylaxis,” first employed to describe tolerance to the toxic effects of foreign proteins after repeated exposure [6], has come in pharmacology to connote a specific loss of response, following prolonged exposure of an effector system to an agonist [7]. In the case of the renal blood supply, where angiotensin is thought to be an important regulatory hormone [8], tachyphylaxis to $A_{II}$ occurs [9]. In an earlier investigation, we described the specificity of renal vascular tachyphylaxis to angiotensin and its offset [5]. However, because of the important role of prostaglandins in renal circulatory homeostasis, and the well-documented effect of $A_{II}$ in promoting renal prostaglandin release [10], it is conceivable that enhanced renal prostaglandin production may have accounted for the observed responses. The results of the present investigation suggest that the recovery phase of renal blood flow and specific desensitization to $A_{II}$ can be dissociated. Although pretreatment with indomethacin attenuated the recovery phase of renal blood flow, it did not prevent specific desensitization to $A_{II}$. Recognizing that a number of interventions employed, e.g., anesthesia, surgery, and placement of catheters into the renal artery, may affect renal vascular response to exogenous $A_{II}$, we selected doses of bolus $A_{II}$, inducing a transient 50 percent reduction in renal blood flow, to adjust for variability between dogs. Our results support the results of previous investigations that vascular effects of $A_{II}$ are accentuated by cyclo-oxygenase inhibitors [11] and extend these observations by dissociating tachyphylaxis from prostaglandin-mediated alterations in vascular tone. Confirmation of tachyphylaxis in our model is evidenced by the sustained response to NE, while bolus $A_{II}$, identical to that inducing a 50 percent reduction in renal blood flow prior to the $A_{II}$ infusion, was without effect. Although prostaglandin levels were not measured nor multiple cyclo-oxygenase inhibitors employed, indomethacin is an agent widely employed for this purpose [10–13], and it produced the expected
response, a fall in renal blood flow in the anesthetized animal [14,15]. Despite cyclo-oxygenase inhibition, a specific loss of response to AII, similar to that of the control group, was demonstrated. Not unlike Aiken’s investigations with isolated celiac and mesenteric arteries [16], our results indicate that at least two mechanisms contribute to loss of vascular response to AII. The first appears to be prostaglandin-dependent and correlates with the recovery phase of renal blood flow, as described by previous investigators [3,10]. The second mechanism, specific desensitization, can be identified following cyclo-oxygenase inhibition in the renal vascular bed.

Following termination of the angiotensin infusion, in three of five dogs in protocols A and B, the time interval (recovery period) required for return of the vasoconstrictor response to bolus AII was determined. Of interest is the fact that the recovery period in the indomethacin group (protocol B) was significantly longer ($p < .03$). Whether this observation reflects a change in receptor affinity [17], decreased receptor turnover [18], altered local angiotensinase activity [19], or evidence that prostaglandins may contribute to recovery from tachyphylaxis cannot be determined from the present study. The sustained response to NE during loss and recovery of the bolus AII response favors a specific event, involving receptor availability or perhaps analogous with beta adrenergic receptor-agonist interaction [20], uncoupling of the AII receptor from a proximate mediator of its effector response. The relatively prolonged recovery times and the short half-life of AII suggest that factors other than a decrease in the bio-phase concentration of AII alone may be operating. Future investigations designed to examine recovery time may prove useful in dissecting the elements responsible for tachyphylaxis.

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