Acetylcholine chloride and renal hemodynamics during postnatal maturation in conscious lambs

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Sener, Alp, and Francine G. Smith. Acetylcholine chloride and renal hemodynamics during postnatal maturation in conscious lambs. J. Appl. Physiol. 87(4): 1296–1300, 1999.—To test the hypothesis that acetylcholine-induced relaxation of the renal artery decreases with postnatal age, we measured parameters of renal hemodynamics before and after aortic suprarenal injection of acetylcholine in conscious, chronically instrumented lambs aged −1 wk (n = 5) and −6 wk (n = 5). Acetylcholine was administered in one of five doses ranging from 0 to 10 mg/kg body wt; doses were administered randomly, in the same volume. There were significant age- and dose-dependent changes in renal vascular resistance after acetylcholine administration, such that the response was greater in 1-wk-old lambs. After the highest dose tested, renal vascular resistance decreased by 13.6 ± 7.3 (SD) mmHg·ml⁻¹·min⁻¹·g kidney wt in 1-wk-old lambs and by 9.1 ± 3.2 mmHg·ml⁻¹·min⁻¹·g kidney wt in 6-wk-old lambs at 35 s. We also observed a transient renal vasoconstriction before the renal vasodilatation in 6-wk-old lambs but not in 1-wk-old animals. These data provide the first age- and dose-dependent effects of exogenous administration of acetylcholine on renal hemodynamics during maturation in conscious animals.

In previous studies on conscious young sheep, we have measured the renal hemodynamic response to nitric oxide synthesis inhibition after the administration of the L-arginine analog N⁵-nitro-L-arginine methyl ester (L-NAME) (20). We observed a marked increase in renal vascular resistance after L-NAME administration, providing the first evidence that nitric oxide normally modulates renal hemodynamics in the conscious young animal under basal conditions (20). In recent experiments, we have further investigated the role of nitric oxide in modulating renal vascular tone during postnatal maturation (21). This capacity of nitric oxide does not appear to be developmentally regulated because, when nitric oxide synthesis is inhibited by a similar extent in both 1- and 6-wk-old conscious lambs by using L-NAME, the resultant increase in renal vascular resistance is similar in both age groups (21). From these observations, we conclude that endogenously produced nitric oxide modulates renal vascular tone to a similar extent in the immediate newborn period compared with later in life.

Interestingly, however, it appears that there may be increased activity of nitric oxide synthase (NOS) early in life. Renal neuronal NOS mRNA is greater at all developmental stages in piglets than that measured in the kidney of the adult pig (24). In addition, the cellular distribution of neuronal NOS mRNA within the kidney is different in newborn rats compared with older animals (8): NOS expression is maximal on postnatal day 6 and declines as maturation proceeds. Taken together, these findings provide evidence to suggest that, for a given stimulus, the production of intrarenally generated nitric oxide may be enhanced in the newborn compared with that observed later in life. To date, however, this has not been investigated, and it forms the basis of our present investigation.

Experiments were carried out to test the hypothesis that acetylcholine-induced renal arterial relaxation decreases with postnatal age in conscious sheep. To test this hypothesis, we measured the renal hemodynamic response to suprarenal aortic injection of acetylcholine chloride into conscious, chronically instrumented lambs aged 1 and 6 wk.

METHODS

Experiments were performed at least 3 days after surgery in conscious, chronically instrumented lambs aged −1 wk (n = 5) and −6 wk (n = 5). Animals were obtained from a local source (Sheep Advisory Service, Innisfail, AB) and housed with their mothers in individual pens in the vivarium of the Health Sciences Center except during surgery and experiments. All surgical and experimental procedures were carried out in accordance with the Guide to the Care and Use of Laboratory Animals.
Experimental Animals provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

Surgical procedures. Surgery was performed on lambs with the use of aseptic techniques, as previously described (7, 23). Briefly, anesthesia was induced with a mask and halothane (3–4%) in oxygen, the trachea was intubated, and anesthesia was maintained by ventilating the lungs with halothane (0.5–1%) in a mixture of nitrous oxide and oxygen (3:1).

The left femoral artery and vein were catheterized (PE-160 catheter, Intramedic, Sparks, MD), and catheters were advanced to the aorta and inferior vena cava for later measurements of arterial and venous pressures and for intravenous infusions. An additional catheter was placed in the right femoral artery and advanced to lie superior to the renal artery for dose arterial injection of acetylcholine chloride during experiments. Catheters were tunneled subcutaneously to exit the lamb on the right and left flanks.

By means of a right flank incision, the right kidney was approached, and a precalibrated ultrasonic flow transducer (model 35-4S, Transonic Systems, Ithaca, NY) was placed around the right renal artery, as previously described (7), for later measurement of renal blood flow. Catheters and the flow transducer cable were contained in pouches on a lamb body jacket (Lomir, Montreal, PQ) for safe storage between experiments. All lambs were able to stand soon after the completion of surgery, at which time they were returned to the vivarium where they were housed with their mothers until the time of the experiment, at least 3 days later. Antibiotics (0.5 mg/kg enrofloxacin; Baytril, Bayer, Etobicoke, ON) were administered intra-arterially at surgery and at 12-h intervals thereafter for 48 h. During this 3-day recovery period, lambs were trained to rest quietly in a supportive sling in the laboratory environment to allow them to become accustomed to their surroundings.

Experimental details. On the day of an experiment the lamb was removed from the vivarium and placed in a supportive sling in the laboratory environment for at least 60 min. An intravenous infusion of 5% dextrose in 0.9% sodium chloride (4.17 ml·kg\(^{-1}\)·min\(^{-1}\)) was started (Baxter, Toronto, ON) to maintain fluid balance during experiments. The flow transducer cable was connected to a flowmeter (model T101, Transonic Systems) for measurement of renal blood flow. The left femoral arterial and venous catheters were connected to pressure transducers (model P23XL, Statham, West Warwick, RI) for measurement of blood pressure and central venous pressure for later calculation of renal vascular resistance. Renal blood flow and pressures were recorded onto a polygraph (model 7, Grass Instruments, West Warwick, RI) and simultaneously to a computer at 200 Hz by using the data-acquisition and -analysis software package CVSOFT (Odessa Systems, Calgary, AB).

Measurements were made for 5 s before and 35 s after dose arterial injection of acetylcholine chloride (Sigma Diagnostics, St. Louis, MO). Acetylcholine chloride was administered at one of five doses (0, 0.01, 0.10, 1.0, and 10 µg/kg), which were obtained by serial dilutions from a stock solution of 3 mg/ml. Each dose was administered in a 0.17-ml volume, three times and at intervals of 5 min; doses were administered in random order.

At the end of the experiments, lambs were killed with a lethal dose of pentobarbitonal sodium. Cather placement was verified by postmortem inspection, and the zero offset of the renal blood flow transducer was determined. Both kidneys were removed and weighed.

Data analyses. For each dose of acetylcholine, the three trials were averaged, and changes in renal blood flow, renal vascular resistance, and blood pressure over the 35 s after injection were determined over 5-s intervals. ANOVA for repeated measures were applied to determine the effect of dose (0–10 µg/kg) and age (1 and 6 wk) on the renal hemodynamic responses to acetylcholine. When the F value was significant, Newman-Keuls multiple-comparison tests were applied to determine where the differences occurred. Significance was accepted at the 95% confidence interval.

RESULTS

Table 1 shows baseline measurements in the two groups of lambs. Renal vascular resistance was higher and renal blood flow was lower in newborns compared with older lambs.

Blood pressure decreased in both age groups of lambs in response to intra-arterial injection of acetylcholine chloride, with the responses being greater at 1-wk than at 6 wk of age as illustrated in Fig. 1. Changes in blood pressure from baseline levels in response to acetylcholine were age dependent (P = 0.038) and dose dependent (P < 0.001); there was also an interaction between age and dose (P = 0.018) and an interaction among age, dose, and time (P = 0.022).

Changes in renal blood flow after administration of acetylcholine chloride were also age dependent (P = 0.002) and dose dependent (P < 0.001) (Fig. 2). There was an interaction between age and dose (P = 0.033) and an interaction among age, dose, and time (P < 0.001) for changes in renal blood flow. At the highest dose tested, there was also a transient decrease in renal blood flow that occurred before the increase in renal blood flow in 6-wk-old lambs; this was not seen in the 1-wk-old animals.

Renal vascular resistance decreased in response to intra-arterial injection of acetylcholine chloride, with the responses being greater at 1 wk than at 6 wk of age as illustrated in Fig. 3. Changes in renal vascular resistance were age dependent (P = 0.019) and dose dependent (P < 0.001). Also, there was an interaction among age, dose, and time (P < 0.001). As with the renal blood flow response, there was a transient increase in renal vascular resistance that was observed only in the 6-wk-old lambs.

There were no effects of vehicle (0 µg/kg) on any of the measured variables.

DISCUSSION

The purpose of the present study in conscious, chronically instrumented lambs was to measure the renal vascular resistance, and blood pressure over the 35 s after injection were determined over 5-s intervals. ANOVA for repeated measures were applied to determine the effect of dose (0–10 µg/kg) and age (1 and 6 wk) on the renal hemodynamic responses to acetylcholine. When the F value was significant, Newman-Keuls multiple-comparison tests were applied to determine where the differences occurred. Significance was accepted at the 95% confidence interval.

Table 1. Baseline measurements in conscious lambs

<table>
<thead>
<tr>
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<th>Newborn Lambs</th>
<th>Older Lambs</th>
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<tbody>
<tr>
<td>Age, days</td>
<td>9±1</td>
<td>45±4*</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>7.2±0.8</td>
<td>10.8±2.1*</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>87±7</td>
<td>90±9</td>
</tr>
<tr>
<td>RBF, ml·min(^{-1})·gkw(^{-1})</td>
<td>2.3±0.6</td>
<td>5.5±1.5*</td>
</tr>
<tr>
<td>RVR, mmHg·ml(^{-1})·min·gkw</td>
<td>33±12</td>
<td>18±5*</td>
</tr>
</tbody>
</table>

Values are means±SD. BP, blood pressure; RBF, renal blood flow; RVR, renal vascular resistance; gkw, gram kidney weight. *P < 0.05 compared with newborn lambs.
hemodynamic responses to acetylcholine chloride during postnatal maturation to determine whether the production of nitric oxide for a given stimulus was greater in the immediate newborn period compared with later in life. Acetylcholine chloride induced a decrease in renal vascular resistance in newborns and in older lambs; this response was attenuated in 6-wk-old animals. Hence, we accept our hypothesis that acetylcholine-induced renal arterial relaxation decreases with postnatal maturation. Interestingly, the highest dose of acetylcholine produced a transient renal vasoconstriction before the renal vasodilatation in 6-wk-old lambs only. Therefore, our data provide the first age-dependent and dose-dependent effects of acetylcholine chloride on renal hemodynamics during maturation in the conscious animal. In addition, these data provide evidence to support the postulate that there are age-dependent changes in the responsiveness of renal vascular endothelial and/or underlying smooth muscle cells to muscarinic-receptor stimulation.

These observations are in keeping with the suggestion that, for a given stimulus, the production of intrarenally generated nitric oxide may be enhanced in the newborn compared with that observed later in life. Using the method cited in Manders et al. (13), we were able to estimate that a similar concentration of drug reached the kidney in 1- and 6-wk-old lambs. This strengthens our findings and confirms our technique as a viable method of delivery of drug to the renal vasculature.

It is important to note, however, that the vasodilatory effects of acetylcholine chloride may be mediated through the endothelial production of agents other than nitric oxide, including endothelial-derived hyperpolarizing factor and prostacyclin. For example, in the isolated perfused rat kidney preconstricted with phenylephrine, acetylcholine-induced vasodilatation was shown to be modulated both by nitric oxide and endothelial-derived hyperpolarizing factor (25). On the other hand, recent evidence suggests that alternative cGMP-independent actions of nitric oxide can explain smooth muscle relaxation and hyperpolarization. For example, Plane et al. (18) showed that endothelium-dependent relaxation to acetylcholine- or nitric oxide-evoked relaxation in rabbit isolated carotid arteries could be mediated by a variety of pathways, including a cGMP-dependent voltage-independent pathway, a cGMP-mediated smooth muscle repolarization, and a cGMP-independent charybotoxin-sensitive smooth muscle repolarization. Therefore, relaxation as well as repolarization to endothelium-derived nitric oxide appears to be mediated by parallel cGMP-dependent and independent pathways (5, 18).

Fig. 1. Dose-dependent effects of acetylcholine on changes (Δ) in blood pressure (BP) from control in 1-wk-old (●) and 6-wk-old lambs (○). A: 0.0 µg/kg. B: 0.01 µg/kg. C: 0.1 µg/kg. D: 1.0 µg/kg. E: 10.0 µg/kg. Values are means ± SD. *P < 0.05 compared with 1-wk-old lambs.

Fig. 2. Dose-dependent effects of acetylcholine on changes in renal blood flow (RBF) from control in 1-wk-old (●) and 6-wk-old lambs (○). A: 0.0 µg/kg. B: 0.01 µg/kg. C: 0.1 µg/kg. D: 1.0 µg/kg. E: 10.0 µg/kg. gkw, Gram kidney weight. Values are means ± SD. *P < 0.05 compared with 1-wk-old lambs.
Our choice of ages for the present study (1 and 6 wk) was based on age-dependent differences in 1) baseline systemic and renal hemodynamics (see also Table 1) and circulating levels of vasoactive factors (15, 19, 22) and 2) the responsiveness of the renal vasculature to other vasoactive agents (16, 23). When these two age groups were compared, the effects of acetylcholine were greater in 1-wk-old lambs. It is possible that the age-dependent effects of acetylcholine on renal hemodynamics resulted from differences in the medullary vs. cortical release of nitric oxide in newborns compared with older lambs. This is based on the observations in adult anesthetized rats that nitric oxide concentration in medullary tissue is higher than that measured in cortical tissue (27). Additional studies are necessary to further investigate this possibility.

One additional notable age-dependent response that we observed in the present study was the transient vasoconstriction that occurred before the vasodilation after administration of the highest dose of acetylcholine chloride to 6-wk-old lambs. Previous studies in coronary arteries (12) and dorsal hand veins (6) of human subjects have provided evidence that acetylcholine produces a biphasic response consisting of a dilation preceded by a constriction. This response is dose dependent such that low doses of acetylcholine produce a vasodilatation, whereas higher doses produce a more predominant vasoconstriction. This finding can be explained by a balance between the effects of acetylcholine acting directly on muscarinic receptors located on underlying smooth muscle cells to cause a vasoconstriction and its effects in eliciting nitric oxide production from endothelial cells. We postulate that there may be age-dependent changes in the localization of muscarinic receptors in the renal vasculature, because the vasoconstriction was not observed in newborn lambs. Evidence to support this postulate is the observation that, in the trachea, high-affinity muscarinic receptors were absent in 1-wk-old piglets compared with that seen in older animals (11); this corresponds to weak tracheal smooth muscle contraction during the first week of life in piglets compared with older animals. Moreover, studies in the myocardium of fetal and adult sheep showed that mRNA for the muscarinic M2-receptor subtype was greatest in fetal heart (4). Also, maximal stimulation of inositol polyphosphatase above basal activity was greatest in fetal myocardiun (4). Relaxation of common carotid and basilar ovine arteries to a nitric oxide donor, nitroglycerine, was also greater in newborn vessels than in adult vessels (17). Correspondingly, baseline levels of cGMP were higher in newborn than in adult segments (17) of carotid and basilar ovine arteries. Taken together, these observations from gene expression, in vitro investigations, and our present study in vivo provide evidence that in a number of vessels, including the renal artery, there is an increased vasodilatory capacity seen soon after birth, compared with later in life.

In conclusion, our experiments provide the first dose-response effects of acetylcholine chloride on renal hemodynamics in conscious, chronically instrumented animals during postnatal maturation. Our results provide new information that there appear to be age-dependent changes in the properties of renal vascular smooth muscle and/or endothelial cells. Moreover, it appears that the capacity of the newborn renal vasculature to release nitric oxide after a given stimulus is greater than that seen later in life.

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REFERENCES


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ACETYLCHOLINE AND RENAL VASCULAR TONE IN NEWBORNS


