Acute Effects of Alpha-Methyldopa on Mean Blood Pressure and Plasma Renin Activity

By Perry V. Halushka and Harry R. Keiser

ABSTRACT

α-Methyldopa significantly lowered mean blood pressure and plasma renin activity in dogs anesthetized with sodium pentobarbital. After unilateral ligation of the right renal vessels and contralateral denervation of the kidney, α-methyldopa significantly lowered mean blood pressure but did not change plasma renin activity. It was concluded that α-methyldopa acutely lowers plasma renin activity via an action mediated through the adrenergic nervous system. However, this decrease in plasma renin activity is not responsible for the decrease in mean blood pressure produced by the drug.

KEY WORDS

anesthesia unilateral renal denervation radioimmunoassay of angiotensin I unilateral nephrectomy dog

α-Methyldopa is an effective antihypertensive agent, but its mechanism of action is not completely understood. It has been postulated (1) that α-methyldopa is converted to α-methylnorepinephrine, a peripheral neurotransmitter less potent than norepinephrine. Recently, evidence that the hypotensive action of α-methyldopa is mediated by the central nervous system has been presented (2–5).

Mohammed et al. (6) found that chronic oral administration of α-methyldopa to one hypertensive patient and a group of normotensive volunteers produced a decrease in plasma renin activity. In a further study (7), stimulation of the renal nerves after chronic oral administration of α-methyldopa to dogs resulted in a significant attenuation of renin release. Therefore, it has been postulated (7) that part of the antihypertensive effect of α-methyldopa is mediated through a decrease in plasma renin activity and that the decrease in plasma renin activity might be partly due to an effect on the intrarenal baroreceptors or the macula densa.

Weidmann et al. (8) found that chronic oral therapy with α-methyldopa significantly lowered plasma renin activity and mean blood pressure in patients with terminal renal failure. They also demonstrated that α-methyldopa is capable of significantly lowering plasma renin activity and mean blood pressure in patients with essential hypertension who have normal or elevated levels of plasma renin activity but not in hypertensive patients who have low levels of plasma renin activity (9).

The purpose of the present study was to determine in dogs if α-methyldopa acutely lowers plasma renin activity and mean blood pressure, if the decrease in plasma renin activity is a direct intrarenal effect or an indirect effect mediated by the adrenergic nervous system, and if the decrease in mean blood pressure is mediated by the decrease in plasma renin activity.

Methods

Two sets of experiments were performed using inbred foxhounds weighing 15–20 kg. In the first set of experiments, 12 dogs were infused with either α-methyldopa or saline via the vertebral artery or the external jugular vein. In the second set of experiments, 11 dogs underwent acute unilateral denervation of the kidney and contralateral ligation of the renal vessels; these dogs were then infused with either α-methyldopa or 0.15M saline via the external jugular vein. In both sets of experiments, the effects of these interventions on plasma renin activity and mean blood pressure were determined.

PROCEDURES

Anesthesia was induced with sodium pentobarbital (30 mg/kg, iv) followed by a constant infusion of sodium pentobarbital (6 mg/kg hour−1) in 5% dextrose in water (12 ml/hour).

In all experiments polyethylene catheters were placed...
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in the right femoral artery and vein for monitoring arterial blood pressure and for the constant infusion of the anesthetic agent. Two catheters were placed in the right external jugular vein: one was positioned close to the right atrium to obtain central venous blood samples for the determination of plasma renin activity, and the second was used for the intravenous administration of α-methyldopa. Mean blood pressure was measured with a Statham P23Db transducer connected to a Hewlett-Packard recorder. Rectal temperatures were monitored throughout the experiment, and body temperature was maintained with a heating pad when necessary.

In 12 dogs, the left vertebral artery was exposed through an anterior incision in the neck. A 25-gauge butterfly needle, which had been bent 90°, was then inserted into the artery. Care was taken to avoid trauma to the vessel or interruption of blood flow. The catheter was kept in place by slight traction and was kept patent by a constant infusion of heparinized saline (4 units/ml) at a rate of 0.5 ml/min.

In 11 other dogs, acute unilateral denervation and contralateral nephrectomy were performed using a trans-abdominal approach. The right kidney was exposed and denervated (2 dogs) or its artery and vein were doubly ligated (9 dogs). The left kidney was then exposed and denervated by cutting all visible renal nerves and painting the artery and vein with liquefied phenol. Immediately after completion of surgery, saline (30-60 ml, iv) was administered.

Thirty minutes after surgery, a 1-hour control infusion of heparinized saline (0.5 ml/min) into either the vertebral artery or the jugular vein was begun. α-Methyldopa (20 mg/kg) dissolved in saline (pH adjusted to 4.5 with In HCl) was infused into either the vertebral artery or the right external jugular vein at the rate of 0.5 ml/min for 1 hour. In the saline-control experiments, an equivalent volume of 0.15m saline (pH 4.5) was administered.

Blood samples (5 ml) for determination of plasma renin activity were taken from the external jugular vein at the middle and the end of the control period and every 30 or 60 minutes thereafter. An equivalent volume of saline was administered after removal of each blood sample. Recordings of mean blood pressure were made when each blood sample was taken. The two values for both plasma renin activity and mean blood pressure obtained during the control period were averaged and used as the control values for that dog. The values obtained in each dog at the various time intervals were compared with the control values using a paired t-test. The Welch-Aspin modification of the t-test (10) was performed on selected values in the denervated and intact groups.

PLASMA RENIN ACTIVITY

Plasma renin activity was determined using modifications of previously described radioimmunooassay procedures (11-13). Briefly, 5-ml venous blood samples were collected in ice-cold Vacutainers containing disodium ethylenediaminetetraacetic acid (EDTA) (1 mg/ml). After centrifugation at 4°C, the plasma was drawn off and stored at −20°C until the time of assay. A 1-ml aliquot of the original sample was adjusted to pH 6 with 1N HCl. Each aliquot was then divided into two equal volumes and incubated for 1 hour at either 37°C or 4°C in the presence of 8-hydroxyquinoline and 2,3-dimercapto-1-propanol in final concentrations of 3.4 mm and 1.6 mm, respectively. After completion of the incubation, duplicate 25-μl aliquots were taken from the tubes and incubated for 18-20 hours at 4°C with an antibody to angiotensin I in a final titer of 1:100,000 and approximately 10,000 counts/min of 118I-angiotensin I (New England Nuclear Corporation). After completion of this incubation, the free 118I-angiotensin I was separated from the bound 118I-angiotensin I by the addition of 1 ml of a suspension of charcoal (0.625 g/100 ml) and dextran (0.0625 g/100 ml) in barbital-sodium buffer (pH 7.4) (14). The mixture was centrifuged at 4,800 g for 10 minutes. The supernatant solution containing the bound 118I-angiotensin I was decanted and counted in a Beckman LS-255 liquid scintillation counter. The scintillation fluid was Biosolve 3 (Beckman) (20 ml) plus Omnifluor (New England Nuclear Corporation) (0.4 g dissolved in 100 ml of toluene) (11).

The standard curve and blank tubes were prepared with a volume of deangiotensinized plasma that was equivalent to that present in the unknown samples (11). Plasma was deangiotensinized using modifications of the method of Boyd et al. (15): 2,3-Dimercapto-1-propanol (20 μliters), 8-hydroxyquinoline solution (100 μliters, 66 mg/ml) and Fuller's earth (120 mg) were added to plasma (10 ml) that had been collected in the usual fashion and kept at 4°C. The plasma was vortexed for 45 seconds and then centrifuged at 30,000 g for 20 minutes. The plasma was divided into aliquots and stored at −20°C. A standard curve was constructed with a computer program that used a log-logit transformation and fit the curve using the least-squares method (16). The minimum detectable amount of angiotensin I present in an aliquot was routinely 5-10 pg. The quality control sample had a mean value of 2.5 ± 0.13 (SE) ng/ml hour−1 after 29 determinations. Plasma renin activity was expressed in ng/ml hour−1 and represented the amount of angiotensin I generated at 37°C minus the amount present in the sample at 4°C.

Results

α-METHYLDOPA IN INTACT DOGS

The effects of a 1-hour infusion of α-methyldopa on plasma renin activity in three groups of dogs anesthetized with sodium pentobarbital are shown in Figure 1. In the four dogs that received α-methyldopa intra-arterially (vertebral artery), the control plasma renin activity was 32.5 ± 4.0 ng/ml hour−1. Plasma renin activity was significantly decreased (P < 0.01) to 12.2 ± 4.4 ng/ml hour−1 by 1 hour after the start of the α-methyldopa infusion and remained significantly decreased (P < 0.01) at 10.4 ± 5.2 ng/ml hour−1 after 1.5 hours. In three of these four dogs, plasma renin activity fell to a value

1 Generously supplied by Merck, Sharp and Dohme, Inc.
Effects of $\alpha$-methyldopa (20 mg/kg) on plasma renin activity (PRA) in dogs anesthetized with sodium pentobarbital; $n = 4$ in each group. I.A. = intra-arterial administration of $\alpha$-methyldopa and I.V. = intravenous administration of the drug.

less than that prior to anesthesia. Plasma renin activity remained significantly decreased for 2 hours after the start of the $\alpha$-methyldopa infusion, but the values had returned to control levels by the third hour. In the four dogs that received $\alpha$-methyldopa intravenously (external jugular vein), the control plasma renin activity was $23.4 \pm 3.4$ ng/ml hour$^{-1}$. Within 30 minutes after the start of the infusion plasma renin activity was significantly decreased ($P < 0.05$) to $16.0 \pm 4.0$ ng/ml hour$^{-1}$; it reached its lowest value of $7.3 \pm 4.0$ ng/ml hour$^{-1}$ ($P < 0.05$) 1.5 hours after the start of the infusion. In two of these four dogs, plasma renin activity fell to a value less than that before anesthesia. Plasma renin activity stayed significantly decreased for 2 hours after the start of the infusion and then returned to control levels by the third hour. In the four dogs that received only saline, plasma renin activity was $36.5 \pm 10.8$ ng/ml hour$^{-1}$ during the control period and did not change significantly throughout the course of the experiment.

Figure 2 shows the effects of $\alpha$-methyldopa on the mean blood pressure in the same dogs considered in Figure 1. In the four dogs that received $\alpha$-methyldopa intra-arterially, the control mean blood pressure was $124 \pm 1$ mm Hg; by 1.5 hours after the start of the infusion the mean blood pressure was significantly decreased ($P < 0.05$) to $108 \pm 4$ mm Hg. The greatest decrease occurred at 5 hours, when the mean blood pressure was $104 \pm 5$ mm Hg ($P < 0.01$). In the four dogs that received $\alpha$-methyldopa intravenously, the control mean blood pressure was $142 \pm 3$ mm Hg, which was significantly ($P < 0.005$) higher than that in the dogs that received $\alpha$-methyldopa intra-arterially. The reason for this difference is unknown. Within 30 minutes after the start of the $\alpha$-methyldopa infusion, the mean blood pressure had decreased significantly ($P < 0.05$) to $137 \pm 3$ mm Hg. Mean blood pressure had fallen to $121 \pm 6$ mm Hg ($P < 0.05$) 1.5 hours after the start of the infusion, after which time it was not significantly decreased from control values. In the four dogs that received the saline, the mean blood pressure was $133 \pm 7$ mm Hg during the control period and, although there was an apparent decrease in the mean pressure at 4 and 5 hours, the changes were not significant.

**ACUTE RENAL DENERVATION**

The effects of a 1-hour intravenous infusion of either $\alpha$-methyldopa or saline on plasma renin activity in dogs that had been subjected to unilateral ligation of the right renal vessels and contralateral denervation of the kidney are shown in Figure 3. In the 6 dogs that received $\alpha$-methyldopa, the control plasma renin activity was $12.1 \pm 1.9$ ng/ml hour$^{-1}$; at no time did plasma renin activity change significantly. To verify that the additional surgical intervention significantly blocked the effect of intravenously administered $\alpha$-methyldopa on plasma renin activity, the Welch-Aspin modification of the t-test was used. The decreases in plasma renin activity 1 and 1.5 hours after the start of intravenous infusion of $\alpha$-methyldopa were compared in the intact dogs and dogs that had been subjected to renal denervation. Renal denervation significantly ($P < 0.025$ and $P < 0.05$) blocked the effect of $\alpha$-methyldopa on plasma renin activity at each of these time intervals. In the 5 dogs that
received saline, plasma renin activity during the control period was 8.8 ± 1.1 ng/ml hour⁻¹ and did not change significantly during the 4-hour observation period. In all 11 dogs, plasma renin activity was significantly less than that in the intact dogs (P < 0.025).

In the acutely denervated dogs that received a-methyldopa intravenously, the mean blood pressure was 121 ± 2 mm Hg during the control period; by 30 minutes after the start of the infusion it had fallen significantly to 114 ± 5 mm Hg (P < 0.05). It continued to decrease to a nadir of 90 ± 5 mm Hg (P < 0.005) at 4 hours (Fig. 4). In the dogs that received only saline, the mean blood pressure was 119 ± 5 mm Hg during the control period, and it did not change significantly during the first 2 hours after the start of the infusion. The mean blood pressure was significantly decreased at the third and fourth hours, 107 ± 5 mm Hg (P < 0.06) and 105 ± 7 mm Hg (P < 0.05), respectively. Slightly more than 50% of the decrease in the mean blood pressure was accounted for by one dog. Nevertheless, the decreases in mean blood pressure at 3 and 4 hours produced by a-methyldopa were significantly greater than those in the saline-control group (P < 0.005 and P < 0.001, respectively). The reason for the decrease in mean blood pressure in the saline-control group is unknown, but it was not due to changes in body temperature.

Discussion

Studies of the effects of various agents on plasma renin activity in anesthetized animals are often difficult because of the initial increase in plasma renin activity after anesthesia and the variable influence that anesthetic agents may have on the physiological and pharmacological control of renin release by the kidneys. However, the fact that plasma renin activity was initially elevated by sodium pentobarbital should not interfere with the interpretation of the results, because it has been shown previously (9) that a-methyldopa appears to be most efficacious in lowering plasma renin activity when it is elevated or normal.

In the present study, the infusion of a-methyldopa into dogs over a period of 1 hour acutely lowered the blood pressure. This finding contrasts with that of a previous report (17) which stated that the acute administration of a-methyldopa in both conscious and anesthetized dogs has no effect on blood pressure. This discrepancy may be due to the doses, usually in the range of 100 mg/kg or greater, used in the previous study (17). Henning and Van Zweiten (2) have found that, in cats anesthetized with chloralose, a dose of a-methyldopa (20 mg/kg) infused into the vertebral artery has a significant hypotensive effect but the same dose infused intravenously does not. Ayitey-Smith and Varma (18) administered a-methyldopa in various doses to normotensive rats anesthetized with urethane-chloralose and found that the "hypotensive effect of 400 mg/kg was not significantly different from that of 25 mg/kg." Thus, it appears that there is a biphasic dose-response relationship between a-methyldopa and its hypotensive effect; possibly at doses in the range of 200 mg/kg to 400 mg/kg, the acute centrally mediated hypotensive response is markedly reduced. Furthermore, at the
higher doses, α-methyldopa may produce a stronger peripheral pressor effect because of its conversion to α-methylnorepinephrine which may antagonize the central hypotensive effect. There is also evidence in the literature that bolus injections of α-methyldopa are not effective (2).

In intact dogs anesthetized with sodium pentobarbital α-methyldopa produced a significant decrease in both plasma renin activity and mean blood pressure; moreover, intravenous and intra-arterial infusions were equipotent. In intact dogs that received α-methyldopa intra-arterially, although the blood pressure remained significantly decreased at 2, 4, and 5 hours, plasma renin activity remained significantly decreased for only 2 hours. In intact dogs that received α-methyldopa intravenously, plasma renin activity also remained significantly decreased for only 2 hours, although the blood pressure in three of the four dogs remained decreased from the control values. It is apparent from the present study that the decreases in mean blood pressure and plasma renin activity parallel each other for only the first 2 hours.

After unilateral nephrectomy and contralateral denervation, α-methyldopa was no longer capable of acutely decreasing plasma renin activity, but mean blood pressure was significantly decreased. Thus, after acute denervation of one kidney and ligation of the contralateral kidney, we were able to selectively block the action of α-methyldopa on the kidney without affecting its action elsewhere in the dog. Although plasma renin activity was not significantly changed by α-methyldopa after renal denervation and ligation, mean blood pressure was significantly decreased. This finding demonstrates that the acute lowering of blood pressure by α-methyldopa does not depend on a decrease in plasma renin activity.

The data from the acutely denervated dogs indicate that α-methyldopa-mediated inhibition of renin release from the kidney requires intact adrenergic innervation. The inhibition of renin release by α-methyldopa in the intact dogs most likely resulted from an inhibition of sympathetic tone to the kidney. Whether the inhibition was mediated through the central nervous system or was the result of an effect on peripheral adrenergic neurons is not known. The data in the present paper and in a previous study (7) tend to rule against any significant effect of α-methyldopa on the intrarenal baroreceptor and the macula densa. Privitera and Mohammed (7) have shown that chronic oral administration of α-methyldopa to dogs significantly inhibits the release of renin in response to renal nerve stimulation. This finding suggests a peripheral adrenergic effect of chronically administered α-methyldopa on the inhibition of renin secretion. However, whether acute administration of α-methyldopa decreases plasma renin activity in the same manner as does chronic administration is unknown. Thus, although the major component of the hypotensive action of α-methyldopa appears to be centrally mediated (2–5), the mechanism by which α-methyldopa acutely lowers plasma renin activity may involve the inhibition of either central or peripheral adrenergic neurons.

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PERRY V. HALUSHKA and HARRY R. KEISER

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