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**Research Article**

Recent studies have demonstrated that the antidiuresis associated with intravenous (i.v.) infusion of the beta adrenergic agonist, isoproterenol (ISO), is mediated by release of endogenous vasopressin. To examine whether beta-adrenergic stimulation causes vasopressin release by a direct cerebral action, ISO was infused into the carotid artery in a dose estimated to equal the amount of catecholamine reaching the cerebral circulation in the i.v. studies. This intracarotid infusion did not alter renal or systemic hemodynamics, urinary osmolality (Uosm) or free-water clearance ($C_{H_2O}$).

Although renal perfusion pressure was maintained constant in all experiments i.v. ISO was consistently associated with a decrease in total peripheral resistance and systemic arterial pressure as cardiac output increased. To investigate whether the decrease in cerebral perfusion pressure with i.v. ISO might be responsible for vasopressin release, the carotid arteries were bilaterally constricted both above and below the carotid sinus to lower carotid perfusion pressure by a mean of 25 mmHg, a decrement comparable to that observed during i.v. ISO. Constriction of the carotid arteries above the carotid sinus did not affect Uosm or $C_{H_2O}$, while constriction below the sinus was associated with an antidiuresis as Uosm increased from 155±25 to 385±58 mosmol/kg ($P < 0.001$) and $C_{H_2O}$ decreased from 1.20 to −0.44 ml/min ($P < 0.001$). This antidiuresis was not significantly different from that observed during i.v. […]



# Mechanism of Stimulation of Vasopressin Release during Beta Adrenergic Stimulation with Isoproterenol

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ABSTRACT Recent studies have demonstrated that the antidiuresis associated with intravenous (i.v.) infusion of the beta adrenergic agonist, isoproterenol (ISO), is mediated by release of endogenous vasopressin. To examine whether beta-adrenergic stimulation causes vasopressin release by a direct cerebral action, ISO was infused into the carotid artery in a dose estimated to equal the amount of catecholamine reaching the cerebral circulation in the i.v. studies. This intracarotid infusion did not alter renal or systemic hemodynamics, urinary osmolality (Uosm) or free-water clearance ($C_{H_2O}$). Although renal perfusion pressure was maintained constant in all experiments i.v. ISO was consistently associated with a decrease in total peripheral resistance and systemic arterial pressure as cardiac output increased. To investigate whether the decrease in cerebral perfusion pressure with i.v. ISO might be responsible for vasopressin release, the carotid arteries were bilaterally constricted both above and below the carotid sinus to lower carotid perfusion pressure by a mean of 25 mmHg, a decrement comparable to that observed during i.v. ISO. Constriction of the carotid arteries above the carotid sinus did not affect Uosm or $C_{H_2O}$, while constriction below the sinus was associated with an antidiuresis as Uosm increased from 155±25 to 385±58 mosmol/kg ($P < 0.001$) and $C_{H_2O}$ decreased from 1.20 to −0.44 ml/min ($P < 0.001$). This antidiuresis was not significantly different from that observed during i.v. ISO. Since these results suggested that changes in autonomic neural tone from arterial baroreceptors are responsible for vasopressin release with i.v. ISO, studies were performed in animals with denervated baroreceptors. While sham-operated animals and animals with bilateral cervical vagotomy showed a reversible antidiuresis with i.v. ISO infusion, dogs with complete denervation of arterial baroreceptors did not show a significant alteration in renal water excretion (Uosm, 187 to 182 mosmol/kg and $C_{H_2O}$, 0.59 to 0.74 ml/min). The results therefore indicate that ISO stimulates vasopressin release by altering baroreceptor tone rather than by a direct central or depressor effect of the catecholamine. These same baroreceptor pathways have been recently shown to be involved in the suppression of vasopressin release with norepinephrine and may well be the common pathway whereby nonosmotic stimuli control vasopressin release.

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## INTRODUCTION

Studies both in man (1) and in experimental animals (2–5) have demonstrated that beta-adrenergic stimulation with the intravenous (i.v.)[1] infusion of isoproterenol (ISO) is associated with a diminution in the excretion of solute-free water. Recent observations have indicated that this antidiuresis is mediated primarily by the release of endogenous vasopressin rather than by an effect of ISO on renal hemodynamics, renal nerves, or the water premeability of the renal tubular epithelium (6). The mechanism whereby this beta-adrenergic agonist causes the release of vasopressin has, however, not been elucidated.

Since both adrenergic and cholinergic agents have been observed to affect the activity of neurosecretory cells in the posterior pituitary (7), it is possible that ISO affects vasopressin release by a direct action on the neurohypophyseal-hypothalamic tract. Alternatively, since the i.v. administration of ISO is associated with alterations in systemic hemodynamics (6), changes in cerebral hemodynamics, such as a decrease in cerebral arterial pressure, could stimulate vasopressin release.

[1] *Abbreviations used in this paper:* $C_{H_2O}$, free-water clearance; FF, filtration fraction; GFR, glomerular filtration rate; ISO, isoproterenol; i.v., intravenous; PAH, *p*-aminohippuric acid; RVR, renal vascular resistance; Uosm, urinary osmolality.

TABLE I

*Effects of Intracarotid ISO on Systemic and Renal Hemodynamics and*

| | Cardiac output | | | Systemic arterial pressure | | | Renal perfusion pressure | | | GFR | | | RVR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control |
| | *liters/min* | | | *mm Hg* | | | *mm Hg* | | | *ml/min* | | | *mm Hg/ml per min* | | |
| Mean | 3.4 | 3.7 | 3.4 | 123 | 123 | 117 | 114 | 115 | 111 | 42.3 | 41.9 | 41.9 | 0.512 | 0.504 | 0.455 |
| SE | ±0.10 | ±0.16 | ±0.18 | ±16 | ±17 | ±16 | ±12 | ±13 | ±12 | ±3.1 | ±3.4 | ±3.0 | ±0.023 | ±0.033 | ±0.037 |
| *P* value | <0.02 | NS | | NS | NS | | NS | <0.05 | | NS | NS | | NS | NS | |

* Precontrol, ISO, and Postcontrol values represent the mean values for six experiments before, during, and after ISO infusion, respectively. The values for renal hemodynamics and electrolyte and water excretion are expressed per kidney.
NS = not significant (*P* value >0.05).

The changes in systemic hemodynamics that occur during the infusion of i.v. ISO could also affect vasopressin release by altering baroreceptor or volume receptor neural tone and thereby decrease parasympathetic afferent stimulation to the central sites of vasopressin release.

The present study was undertaken to investigate whether any of the above mechanisms are responsible for the increased release of vasopressin during beta-adrenergic stimulation with i.v. ISO. The results of the present studies did not provide evidence for a direct effect of either the catecholamine or cerebral arterial pressure on vasopressin release. The results, however, did indicate that a change in baroreceptor neural tone is primarily responsible for the antidiuresis associated with the i.v. infusion of ISO.

## METHODS

30 experiments were performed in 19 mongrel dogs of either sex weighing 20–30 kg. Food was withheld from these animals for 18 h before study, but water was allowed ad lib. On the day of study the animals were anesthetized with i.v. pentobarbital (30 mg/kg), intubated, and ventilated with a Harvard respirator. Light anesthesia was maintained throughout the experiment by the intermittent administration of pentobarbital. After induction of anesthesia all animals received 5 mg of deoxycorticosterone acetate in oil intramuscularly. A solution of 2.5% glucose and water was then infused through a catheter in a foreleg vein at 20 ml/min for 50 min during which time the following surgery was performed. Polyethylene catheters were placed in both ureters and renal veins through bilateral flank incisions by a retroperitoneal approach. In 11 animals an adjustable Blalock clamp was placed around the aorta above the origin of both renal arteries. In all animals a catheter was inserted into the aorta via the brachial artery for the continuous measurement of arterial pressure with Statham transducers (Statham Instruments, Inc., Oxnard, Calif.) and a direct writing Gilson recorder (Gilson Medical Electronics, Inc., Middleton, Wis.). In the animals with the Blalock clamp around the aorta, the arterial pressure was also measured in the aorta below the clamp via a catheter inserted in the femoral artery. In the animals which received ISO, a catheter was inserted into the right atrium via the jugular vein to inject indocyanine green dye for determination of cardiac output by the dye-dilution method using methods and

TABLE II

*Effects of Decreased Carotid Arterial Pressure on Systemic and*

| | Systemic arterial pressure | | | Carotid arterial pressure | | | GFR | | | RVR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-control | Decreased CAP | Post-control | Pre-control | Decreased CAP | Post-control | Pre-control | Decreased CAP | Post-control | Pre-control | Decreased CAP | Post-control |
| | *mm Hg* | | | *mm Hg* | | | *ml/min* | | | *mm Hg/ml per min* | | |
| A. Above carotid sinus‡ | | | | | | | | | | | | |
| Mean | 142 | 139 | 136 | 142 | 117 | 136 | 46.6 | 43.9 | 45.3 | 0.407 | 0.396 | 0.387 |
| ±SE | ±10 | ±12 | ±13 | ±10 | ±11 | ±13 | ±3.1 | ±2.9 | ±3.8 | ±0.017 | ±0.015 | ±0.016 |
| *P* value | NS | NS | | <0.001 | <0.005 | | <0.005 | NS | | NS | NS | |
| B. Below carotid sinus§ | | | | | | | | | | | | |
| Mean | 153 | 180 | 147 | 147 | 122 | 140 | 37.3 | 36.0 | 34.5 | 0.738 | 0.977 | 0.721 |
| ±SE | ±3 | ±5 | ±7 | ±2 | ±2 | ±5 | ±2.9 | ±2.5 | ±1.9 | ±0.074 | ±0.081 | ±0.078 |
| *P* value | <0.005 | <0.025 | | <0.001 | <0.01 | | NS | NS | | <0.001 | <0.001 | |

* Precontrol, Decreased CAP, Postcontrol values represent periods before, during, and after decreased carotid arterial pressure, respectively.
‡ The results are the mean values from six experiments; the values for renal hemodynamics and electrolyte and water excretion are expressed per kidney.
§ The results are the mean values from five experiments.

*Electrolyte and Water Excretion in Dogs Undergoing a Water Diuresis**

| FF | | | Urinary sodium excretion | | | Urinary potassium excretion | | | $C_{H_2O}$ | | | Uosm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control |
| | | | *μeq/min* | | | *μeq/min* | | | *ml/min* | | | *mosmol/kg $H_2O$* | | |
| 0.304 | 0.294 | 0.272 | 18 | 21 | 18 | 25 | 27 | 27 | 3.74 | 3.38 | 3.34 | 68 | 80 | 80 |
| ±0.022 | ±0.026 | ±0.031 | ±5 | ±6 | ±5 | ±2 | ±2 | ±2 | ±0.52 | ±0.59 | ±0.53 | ±6 | ±7 | ±7 |
| NS | NS | | NS | NS | | NS | NS | | <0.02 | NS | | <0.001 | NS | |

instruments as previously described (8). To allow infusion of ISO directly into the cerebral circulation a 23-gauge needle was placed in the left carotid artery and maintained patent by infusion of solution of 2.5% glucose and water at 0.5 ml/min. In some animals Hoffman screw-clamps were placed around both carotid arteries either immediately below or above the region of the carotid sinus to allow lowering of cerebral arterial pressure. When the clamp was placed below the level of the carotid sinus, it was above the level of the thyrocarotid junction. In these animals a 25-gauge catheter was threaded above the screw-clamp to record cerebral perfusion pressure. In animals in which the screw-clamp was placed below the carotid sinus, bilateral cervical vagotomy was performed to avoid any reflex changes in vasopressin release mediated by the aortic arch baroreceptors. In another group of animals baroreceptor denervation was undertaken by performing bilateral cervical vagotomy or a combination of carotid sinus denervation and bilateral cervical vagotomy. Denervation of the carotid sinus was performed by stripping and cutting the nerves surrounding the carotid artery at its bifurcation and applying 100% alcohol to this site. Bilateral cervical sham-operations were performed in another group of animals by dissection and manipulation of the carotid arteries, vagal nerves, and the other surrounding tissues.

After completion of surgery, an i.v. infusion of 0.9% saline (0.5 ml/min) was begun through a catheter in a foreleg vein which contained sufficient inulin and *p*-aminohippuric acid (PAH) to maintain blood levels of these substances between 15 and 25 and 1 and 3 mg/100 ml, respectively. After 1 liter of 2.5% glucose and water had been infused, the rate of the infusion was decreased to 4 ml/min above urine flow. After completion of surgery an additional 600–1,000 ml of 2.5% glucose and water was administered over 60–90 min, then the infusion rate was decreased again to 4 ml/min above urine flow. Several animals with denervated baroreceptors did not have a water diuresis and therefore were not used in the study. In the animals in which a diuresis was obtained, the experiments were started when stabilization of urine flow occurred. Urine was collected at 5-min intervals throughout the experiment and arterial and renal venous blood samples were collected at the midpoint of alternate collections of urine. Cardiac output was measured every third period during the experiment. The experiments were carried out according to the following protocols.

*Intracarotid administration of ISO.* The dose of ISO infused into the carotid artery ranged from 0.004 to 0.009 μg/kg per min. This dose was chosen to deliver a concentration of the drug which was similar to that reaching

*Renal Hemodynamics in Dogs Undergoing a Water Diuresis**

| FF | | | Urinary sodium excretion | | | Urinary potassium excretion | | | $C_{H_2O}$ | | | Uosm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-control | Decreased CAP | Post-control | Pre-control | Decreased CAP | Post-control | Pre-control | Decreased CAP | Post-control | Pre-control | Decreased CAP | Post-control | Pre-control | Decreased CAP | Post-control |
| | | | *μeq/min* | | | *μeq/min* | | | *ml/min* | | | *mosmol/kg $H_2O$* | | |
| 0.264 | 0.247 | 0.248 | 16 | 16 | 17 | 34 | 36 | 39 | 2.93 | 3.26 | 3.12 | 78 | 73 | 80 |
| ±0.016 | ±0.017 | ±0.018 | ±2 | ±2 | ±2 | ±4 | ±4 | ±4 | ±0.30 | ±0.24 | ±0.21 | ±6 | ±5 | ±5 |
| <0.01 | NS | | NS | NS | | NS | <0.05 | | NS | NS | | NS | <0.005 | |
| 0.334 | 0.387 | 0.328 | 34 | 26 | 31 | 24 | 27 | 28 | 1.20 | 0.44 | 0.90 | 155 | 385 | 194 |
| ±0.021 | ±0.015 | ±0.017 | ±8 | ±7 | ±7 | ±3 | ±4 | ±4 | ±0.28 | ±0.12 | ±0.33 | ±25 | ±58 | ±39 |
| <0.05 | <0.02 | | NS | NS | | NS | NS | | <0.001 | <0.001 | | <0.001 | <0.001 | |

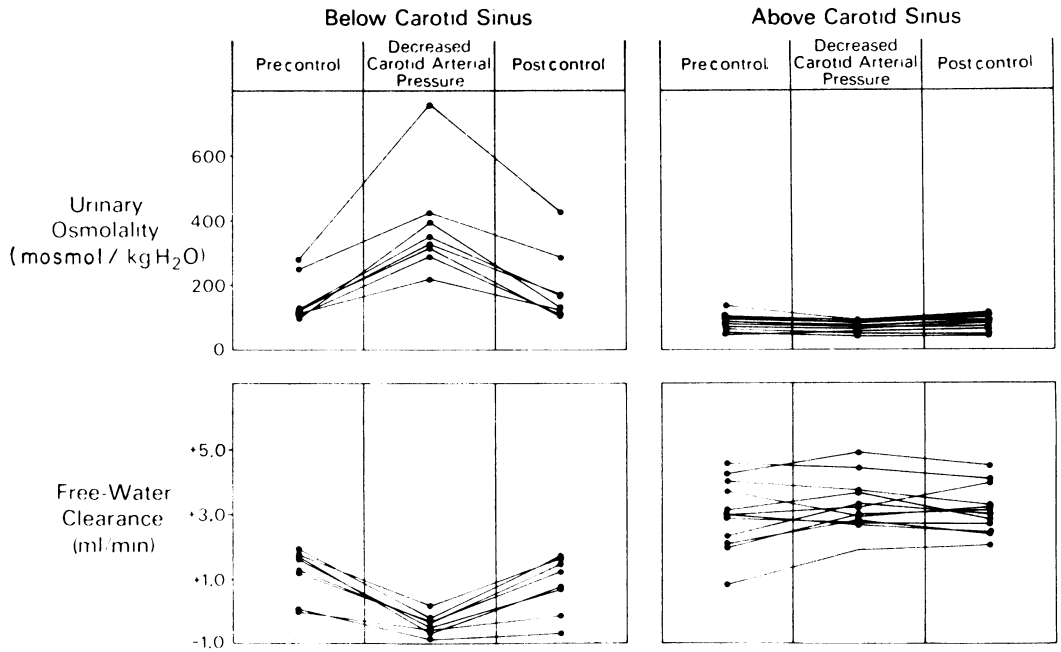


FIGURE 1 Effect of decreased cerebral perfusion on Uosm (above) and $C_{H_2O}$ (below) in dogs with carotid constriction below the carotid sinus (left) and above the carotid sinus (right). Each point represents the mean value of three to five urine collections from a single kidney. The mean level of carotid and systemic arterial pressure for each period are shown in Table II.

the cerebral circulation during previous studies in which i.v. ISO was associated with a decrease in renal water excretion (6). Three to five control periods were collected and then the ISO infusion into the carotid artery was started. After an equilibration period of 30 min, three to five experimental urine collections were made. The infusion of ISO was then discontinued and after an equilibration period of 20–30 min, three to five postcontrol urine collections were made.

*Decreased carotid arterial pressure.* After three to five control urine collections, pressure in the carotid arteries was bilaterally decreased by adjusting the screw-clamps which were situated either above or below the carotid sinus. After a 30-min equilibration period, three to five experimental urine collections were made. The screw-clamps were then released and after a 20–30-min equilibration period, three to five postcontrol urine collections were made.

*i.v. administration of ISO.* In these studies i.v. ISO (0.018–0.036 μg/kg per min) was infused into three groups of dogs: (*a*) sham-operated animals, (*b*) animals with bilateral cervical vagotomy, and (*c*) the animals with both bilateral cervical vagotomy and carotid sinus denervation. Except that the drug was infused into a peripheral vein rather than the carotid artery, the experimental protocol was the same as in the studies with intracarotid infusion of ISO. Arterial perfusion pressure to the kidneys was maintained constant throughout the experiment by adjustment of the suprarenal aortic clamp.

The analytical procedures and calculations used in the present experiments have been referred to elsewhere (9). The following abbreviations will be used: glomerular fil-

TABLE III

*Effects of i.v. ISO on Systemic and Renal Hemodynamics and*

| | Cardiac output | | | Systemic arterial pressure | | | Renal perfusion pressure | | | GFR | | | RVR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control |
| | *liters/min* | | | *mm Hg* | | | *mm Hg* | | | *ml/min* | | | *mm Hg/ml per min* | | |
| Mean | 2.9 | 5.2 | 3.2 | 150 | 134 | 153 | 120 | 120 | 121 | 47.8 | 46.0 | 50.6 | 0.420 | 0.515 | 0.426 |
| ±SE | ±0.4 | ±0.3 | ±0.7 | ±7 | ±7 | ±7 | ±4 | ±4 | ±5 | ±2.5 | ±2.8 | ±3.8 | ±0.053 | ±0.057 | ±0.057 |
| *P* value | <0.001 | | <0.02 | <0.001 | | <0.001 | NS | | NS | NS | | <0.05 | <0.005 | | <0.005 |

* See Table I for abbreviations.

tration rate (GFR), filtration fraction (FF), renal vascular resistance (RVR), free-water clearance ($C_{H_2O}$), and urinary osmolality (Uosm).

## RESULTS

*Intracarotid ISO studies (Table I).* These studies were performed to examine whether an increase in carotid arterial concentration of ISO, similar to that estimated to occur during previous i.v. studies (6), is associated with an effect on renal water excretion in the absence of significant changes in systemic hemodynamics. In these studies a small but not statistically reversible increase in cardiac output occurred and systemic arterial pressure was not significantly changed. These results suggested that there were no significant systemic hemodynamic effects of the drug when it was infused into the carotid artery over the dosage range (0.004–0.009 μg/kg per min) used. The mean values for the six experiments including effects on renal hemodynamics and electrolyte and water excretion are shown in Table I. The intracarotid administration of ISO did not significantly alter GFR, RVR, or FF, nor was it associated with any changes in electrolyte excretion. Uosm increased slightly but significantly from 68±6 to 80±7 mosmol/kg $H_2O$ during ISO infusion, but did not decrease significantly after cessation of the infusion. Similarly, $C_{H_2O}$ decreased slightly during the infusion of ISO but did not return to the control level after the infusion was discontinued.

*Decreased carotid arterial pressure studies (Table II, Fig. 1).* In order to examine whether decreases in carotid arterial pressure, such as occur during the i.v. administration of ISO, are associated with any changes in renal water excretion, carotid arterial pressure was decreased bilaterally by adjusting Hoffman screw-clamps. In the first group of animals, the screw-clamps were placed above the carotid sinus. The mean values for the six experiments including effects on renal hemodynamics, electrolyte and water excretion are shown in Table II *A*. There were no significant changes in systemic arterial pressure as carotid arterial pressure was decreased from 142±10 to 117±11 mmHg ($P < 0.001$) and then increased to 136±11 mmHg ($P < 0.005$). This maneuver was not associated with any significant or reversible alterations in GFR, RVR, FF, or electrolyte excretion. There was no evidence for a direct effect of decreased carotid arterial pressure to release vasopressin as Uosm was 78±6 before, 73±5 during, and 80±5 mosmol/kg $H_2O$ after bilateral carotid constriction, respectively. $C_{H_2O}$ was 2.93±0.30, 3.26±0.24, and 3.12±0.21 ml/min during the same periods.

The mean values for the five experiments in which the carotid arteries were constricted below the carotid sinus are shown in Table II *B*. In these experiments as carotid arterial pressure was decreased from 147±2 to 122±2 mmHg ($P < 0.001$) and increased to 140±5 ($P < 0.01$), systemic arterial pressure increased from 153±3 to 180±5 mmHg ($P < 0.005$) and decreased to 147±7 mmHg ($P < 0.025$) after the carotid constriction was released. This maneuver did not significantly alter GFR; however, RVR and FF increased significantly with carotid constriction and decreased to control levels when the constriction was released. Decreased carotid arterial pressure was not associated with any significant changes in urinary sodium or potassium excretion. In spite of the significant increase in renal perfusion pressure, Uosm increased from 155±25 to 385±58 mosmol/kg $H_2O$ ($P < 0.001$) with carotid constriction below the carotid sinus and decreased to 194±39 mosmol/kg $H_2O$ ($P < 0.01$) when the constriction was released. At the same time, $C_{H_2O}$ decreased from 1.20±0.28 to −0.44± 0.12 ml/min ($P < 0.001$) then increased to 0.90±0.33 ml/min ($P < 0.001$). The different effects of bilateral carotid artery constriction above and below the carotid sinus on renal water excretion are plotted in Fig. 1.

*i.v. ISO studies (Tables III–V and Fig. 2).* These studies were performed to examine whether intact cervical parasympathetic pathways are necessary for demonstration of the antidiuretic effect of i.v. ISO. The results of all experiments in sham-operated animals including systemic and renal hemodynamics and electrolyte and water excretion are shown in Table III. The sham-operated animals responded to the i.v. infusion of ISO in

*Electrolyte and Water Excretion in Sham-Operated Dogs**

| FF | | | Urinary sodium excretion | | | Urinary potassium excretion | | | $C_{H_2O}$ | | | Uosm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-contro |
| | | | *μeq/min* | | | *μeq/min* | | | *ml/min* | | | *mosmol/kg $H_2O$* | | |
| 0.281 | 0.358 | 0.288 | 4 | 5 | 6 | 16 | 15 | 18 | 1.30 | 0.16 | 1.90 | 116 | 272 | 97 |
| ±0.019 | ±0.020 | ±0.018 | ±1 | ±1 | ±1 | ±1 | ±2 | ±2 | ±0.19 | ±0.11 | ±0.30 | ±12 | ±32 | ±8 |
| <0.005 | | <0.001 | NS | | <0.05 | NS | | NS | <0.001 | | <0.001 | <0.001 | | <0.001 |

TABLE IV

*Effects of i.v. ISO on Systemic and Renal Hemodynamics and*

| | Cardiac output | | | Systemic arterial pressure | | | Renal perfusion pressure | | | GFR | | | RVR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control |
| | *liters/min* | | | *mm Hg* | | | *mm Hg* | | | *ml/min* | | | *mm Hg/ml per min* | | |
| | | | | Systemic arterial | | | Renal perfusion | | | | | | | | |
| Mean | 4.5 | 7.3 | 4.7 | 143 | 127 | 139 | 115 | 111 | 112 | 51.3 | 53.1 | 51.9 | 0.330 | 0.374 | 0.333 |
| ±SE | ±0.07 | ±0.75 | ±0.05 | ±13 | ±12 | ±8 | ±4 | ±2 | ±2 | ±5.7 | ±6.8 | ±6.6 | ±0.039 | ±0.057 | ±0.040 |
| *P* value | | | | <0.02 | NS | | NS | NS | | NS | NS | | NS | NS | |

* The results are the mean values from four experiments. Since Cardiac output measurements were made only in two of these experiments statistics are not shown for this parameter.
See Table I for abbreviations.

a manner similar to that described in previous studies in the intact dog (6). i.v. ISO infusion was associated with a significant increase in Uosm from 116±12 to 272±32 mosmol/kg $H_2O$ ($P < 0.001$) which decreased to 97±8 mosmol/kg $H_2O$ ($P < 0.001$) after cessation of the infusion. At the same time, $C_{H_2O}$ decreased from 1.30±0.19 to 0.16±0.11 ml/min ($P < 0.001$) during the infusion and increased to 1.90±0.30 ml/min ($P < 0.001$) after the ISO was discontinued. Cardiac output increased from 2.9±0.4 to 5.2±0.3 liters/min ($P < 0.001$) with ISO and decreased to 3.2±0.7 liters/min ($P < 0.02$) after cessation of the infusion. i.v. ISO was also associated with a decrease in mean systemic arterial pressure from 150±7 to 134±7 ($P < 0.001$) which increased to 153±7 mmHg ($P < 0.001$) after the infusion was discontinued. Renal perfusion pressure was maintained constant throughout the experiment by adjustment of the suprarenal aortic clamp. Significant and reversible changes in RVR and FF occurred during the i.v. infusion of ISO; however, no reversible alterations in GFR or electrolyte excretion were observed.

The effects of i.v. ISO on systemic and renal hemodynamics, electrolyte and water excretion in dogs in which bilateral cervical vagotomy had been performed are shown in Table IV. In these vagotomized animals, i.v. ISO was associated with alterations in renal water excretion which were not significantly different from those seen in the studies in sham-operated animals. Uosm increased from 83±21 to 340±60 mosmol/kg $H_2O$ ($P < 0.005$) during the ISO infusion and decreased to 102±27 mosmol/kg $H_2O$ ($P < 0.01$) after cessation of the infusion. At the same time, $C_{H_2O}$ decreased from 3.36±0.58 to −0.04±0.22 ml/min ($P < 0.001$) and increased to 2.67±0.66 ml/min ($P < 0.01$). The systemic hemodynamic alterations associated with i.v. ISO were also similar to those observed in the sham-operated animals studies. There were no reversible changes in GFR, RVR, FF, or electrolyte excretion associated with ISO in this group of animals.

The effects of i.v. ISO on systemic and renal hemodynamics and electrolyte and water excretion in all the dogs with denervated aortic and carotid baroreceptors are shown in Table V. In contrast to the studies in sham-operated and vagotomized dogs, ISO was not associated with any significant alterations in renal water excretion in dogs with denervated aortic and carotid baroreceptors. Uosm was 187±7 before, 182±15 during, and 177±17 mosmol/kg $H_2O$ after the i.v. infusion of ISO, respectively, as $C_{H_2O}$ was 0.59±0.18, 0.74±0.22, and 0.78±0.31 ml/min during the same periods. Since denervation of the baroreceptors alone is associated with an antidiuresis (10), these studies had to be performed

TABLE V

*Effects of i.v. ISO on Systemic and Renal Hemodynamics and*

| | Cardiac output | | | Systemic arterial pressure | | | Renal perfusion pressure | | | GFR | | | RVR | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-contro |
| | *liters/min* | | | *mm Hg* | | | *mm Hg* | | | *ml/min* | | | *mm Hg/ml per min* | | |
| Mean | 3.3 | 3.8 | 2.7 | 140 | 112 | 130 | 115 | 104 | 103 | 32.3 | 32.5 | 32.3 | 0.811 | 0.740 | 0.747 |
| ±SE | ±0.6 | ±0.6 | ±0.5 | ±12 | ±14 | ±15 | ±6 | ±10 | ±10 | ±3.7 | ±3.8 | ±4.0 | ±0.096 | ±0.084 | ±0.091 |
| *P* value | <0.02 | <0.005 | | <0.01 | <0.005 | | NS | NS | | NS | NS | | <0.02 | NS | |

* See Table I for abbreviations.

*Electrolyte and Water Excretion in Vagotomized Dogs Undergoing a Water Diuresis**

| FF | | | Urinary s9dium excretion | | | Urinary potassium excretion | | | $C_{H_2O}$ | | | Uosm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control |
| | | | *eq/min* | | | *eq/min* | | | *ml/min* | | | *mosmol/kg $H_2O$* | | |
| 0.231 | 0.259 | 0.231 | 15 | 14 | 8 | 24 | 26 | 22 | 3.36 | −0.04 | 2.67 | 83 | 340 | 102 |
| ±0.026 | ±0.026 | ±0.023 | ±7 | ±4 | ±2 | ±2 | ±4 | ±1 | ±0.58 | ±0.22 | ±0.66 | ±21 | ±60 | ±27 |
| NS | <0.01 | | NS | NS | | NS | NS | | <0.001 | <0.01 | | <0.005 | <0.01 | |

in animals in which the Uosm was above the minimal level (50–100 mosmol/kg). It seems unlikely, however, that this somewhat higher level of control Uosm obscured the detection of an antidiuresis. The studies with carotid occlusion below the carotid sinus were also performed in animals with a similar control level of Uosm and yet an antidiuresis was readily demonstrable. Moreover, studies were performed in some sham-operated animals with less than maximally dilute urine and an antidiuresis was readily observed (Table III). The effects on systemic hemodynamics in this group of animals with carotid and aortic arch baroreceptor denervation were similar to those seen in the sham-operated and vagotomized animals. There were also no reversible effects on GFR, RVR, FF, or electrolyte excretion. The hematocrit values in the baroreceptor-denervated group before (41±6%), during (41±6%), and after ISO (40±6%) were not significantly different. During the same periods the hematocrit values in the sham-operated groups were 45±6, 49.5±7, and 45±6%. These latter values were only significantly different from hematocrit values in the baroreceptor-denervated group during the infusion of ISO. This increase in hematocrit and the concomitant increase in FF do not, however, seem to account for the different effects of ISO on Uosm and $C_{H_2O}$ in the sham-operated and baroreceptor-denervated groups since we have previously demonstrated that the same increase in hematocrit and filtration fraction occurs in hypophysectomized animals during ISO even though very little effect on renal water excretion is observed (6). It seems likely that the absence of an increase in filtration fraction and hematocrit in the animals with denervated baroreceptors relates to the absence of increased sympathetic tone during the vasodepressor effect of ISO. In support of this likelihood is the finding that the same dose of ISO produced a greater mean decrement in blood pressure in the baroreceptor-denervated dogs than in the sham-operated dogs (−28 vs. −16 mmHg).

The plasma osmolalities in the sham-operated animals were not significantly different before, during and after ISO (259±8, 257±7, and 253±7 mosmol/kg). During the same periods the plasma osmolalities in the baroreceptor-denervated animals progressively decreased by a modest degree from 241±12, 237±12, and 232±13 mosmol/kg. Such diminished plasma osmolalities are a consistent finding in the anesthetized animals undergoing a water diuresis. It seems unlikely, however, that any differences in plasma osmolality accounted for the different effects of ISO on Uosm and $C_{H_2O}$ in the sham-operated and baroreceptor-denervated animals. In support of this interpretation is the finding that in several

*Electrolyte and Water Excretion in Dogs with Denervated Baroreceptors**

| FF | | | Urinary sodium excretion | | | Urinary potassium excretion | | | $C_{H_2O}$ | | | Uosm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control | Pre-control | ISO | Post-control |
| | | | *μeq/min* | | | *μeq/min* | | | *ml/min* | | | *mosmol/kg $H_2O$* | | |
| 0.345 | 0.351 | 0.343 | 18 | 17 | 14 | 25 | 25 | 24 | 0.59 | 0.74 | 0.78 | 187 | 182 | 177 |
| ±0.011 | ±0.012 | ±0.015 | ±4 | ±5 | ±4 | ±4 | ±4 | ±4 | ±0.18 | ±0.22 | ±0.31 | ±17 | ±15 | ±17 |
| NS | NS | | NS | <0.02 | | NS | NS | | NS | NS | | NS | NS | |

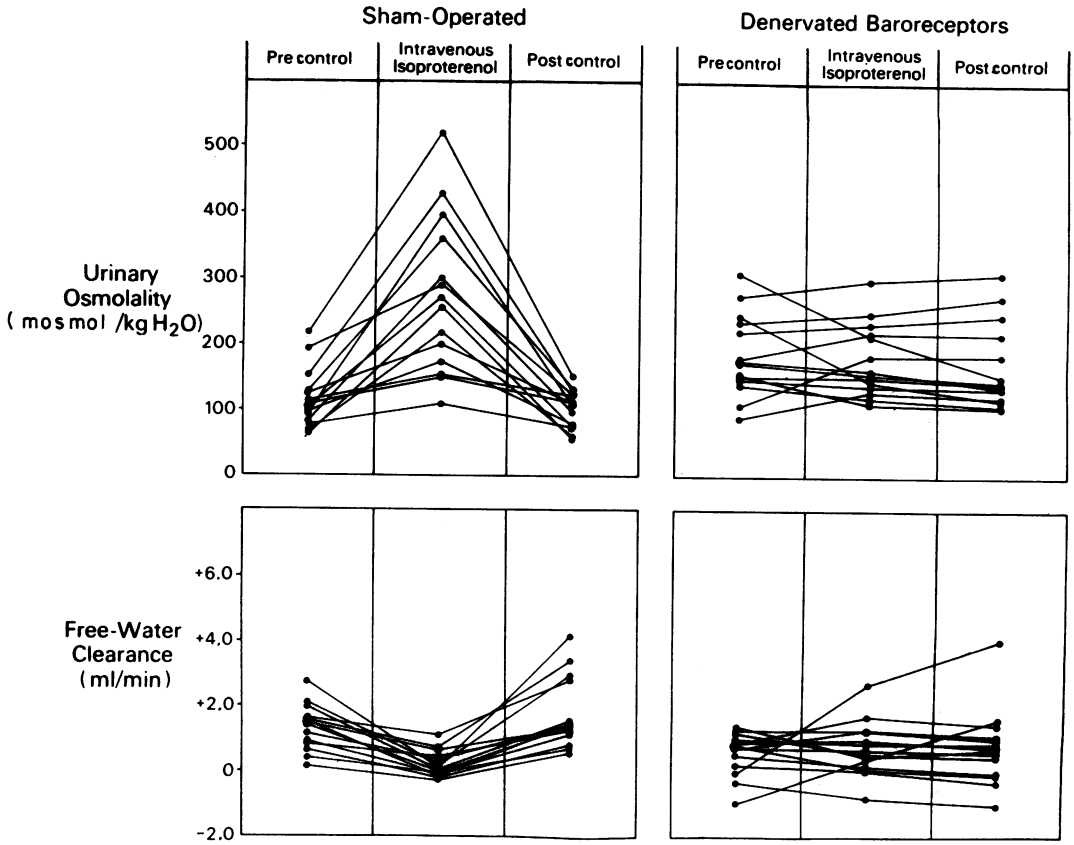


FIGURE 2 Effect of i.v. ISO on Uosm (above) and $C_{H_2O}$ (below) in animals with cervical sham operation (left) and denervation of baroreceptors (right). Each point represents the mean value of three to five urine collections from a single kidney.

experiments the plasma osmolalities were actually lower in experiments in the sham-operated dogs than in dogs with denervated baroreceptors and yet the antidiuresis with ISO was observed only in the sham-operated animals. The different effect of beta-adrenergic stimulation with i.v. ISO on renal water excretion in the sham-operated animals and animals with denervated baroreceptors is shown in Fig. 2.

## DISCUSSION

The results of several investigations indicate that alpha- and beta-adrenergic stimulation exert profound and opposing effects on renal water excretion (1–6, 11–13). In both man and animals alpha-adrenergic stimulation with i.v. norepinephrine is consistently associated with a water diuresis (5, 11–13) while beta-adrenergic stimulation with i.v. ISO is associated with an antidiuresis (1, 2, 4, 6). These opposing effects of beta- and alpha-adrenergic stimulation on water excretion have been dissociated from changes in renal perfusion pressure, filtration rate, renal innervation, and solute excretion and are not observed in animals in which the source of release of vasopressin from the posterior pituitary has been removed (6, 11). Therefore, although some in vitro studies suggest an interaction between the effect of catecholamines and vasopressin on osmotic water movement and intracellular concentrations of 3′5′-adenosine monophosphate (cyclic AMP) (14, 15), the predominant in vivo effect of catecholamines on water excretion appears to be mediated by alterations in the endogenous release of vasopressin.

The present studies were undertaken to investigate the mechanism whereby beta-adrenergic stimulation with ISO stimulates the release of vasopressin. Since catecholamines have been shown to influence neurosecretory activity of cells in the supraoptic nuclei of the hypothalamus (7), the possibility existed that the effect of ISO to increase vasopressin release was a direct intracerebral effect. Such a conclusion could not, however, be drawn from previous studies during the i.v. infusion of ISO because of the concomitant alterations in systemic hemodynamics which occurred in these studies (6). In the present investigation ISO was infused directly into the carotid artery in a concentration that approximated

those achieved in the cerebral circulation during the previous studies in which an antidiuresis occurred when ISO was infused intravenously (6). Since this intracarotid infusion was not associated with alterations in systemic hemodynamics, any antidiuretic effect observed would be most likely related to either some direct or intracerebral vascular effect of beta-adrenergic stimulation on vasopressin release. The failure to demonstrate such an effect in the present studies, however, suggested that an increase in the cerebral arterial concentration of the catecholamine is not primarily responsible for the antidiuresis associated with i.v. ISO. These results thus suggested that some extracerebral consequence of beta-adrenergic stimulation may initiate a reflex leading to increased release of vasopressin.

The alterations in systemic hemodynamics which occur during the i.v. administration of ISO therefore seemed a possible initiating event for such a reflex. Since in the present and previous studies (6) the capacity of beta-adrenergic stimulation with ISO to decrease total peripheral resistance was more profound than the enhancement of cardiac output, a fall in systemic arterial pressure was a consistent accompaniment of the antidiuretic effect. Although renal arterial pressure was maintained constant, a diminution in cerebral perfusion pressure did occur during the i.v. infusion of ISO and therefore could have been involved in the increased release of vasopressin. Some support for this possibility is derived from the finding that diminishing cerebral perfusion pressure by carotid artery occlusion is known to be associated with an antidiuresis (16) and from the fact that investigators using bioassay techniques also have reported a concomitant increase in the circulating level of vasopressin during the cartoid artery occlusion (17, 18). There does not, however, seem to be uniform agreement as to whether such an effect of carotid artery occlusion is mediated by indirectly altering afferent baroreceptor tone or whether a decrease in cerebral arterial pressure per se directly stimulates vasopressin release. Although the interruption of afferent vagal pathways is known to stimulate vasopressin release (10), the effect of carotid occlusion to increase bioassayable titers of vasopressin has not been demonstrable in animals with intact vagi (19). While Share and Levy (17) reported that carotid sinus denervation abolishes the effect of carotid occlusion to increase vasopressin titers, Lemaire, Mazer, and Allegrini (20) have reported increased plasma antidiuretic activity and decreased urine flow after carotid occlusion in animals with carotid sinus denervation. Moreover, Perlmutt (16) observed an antidiuresis only when the carotid artery was occluded below the thyrocarotid junction and he observed no effect when the carotid artery was occluded between the thyrocarotid junction and the carotid sinus. Taken together, therefore, the pathway whereby beta-adrenergic stimulation might stimulate vasopressin release was not readily apparent from the results of these previous studies with carotid occlusion. Moreover, it is also possible that total occlusion of the carotid artery might initiate a different reflex than that observed when cerebral perfusion pressure is decreased by 15–25 mmHg.

Studies were therefore performed to examine whether lowering cerebral arterial pressure by constricting both carotid arteries above the level of the carotid sinus, and thus above the level of the known extracerebral baroreceptors, was associated with an alteration in urine flow in animals undergoing a water diuresis. The degree of constriction was such that the arterial pressure above the carotid constriction was decreased to a level comparable to that observed during beta-adrenergic stimulation during i.v. ISO administration. This maneuver was not associated with an alteration in either Uosm or the rate of solute-free water excretion. These results are compatible with our recent observation that increasing cerebral perfusion pressure in animals with denervated baroreceptors by a pump-perfusion method was not associated with an effect on renal water excretion (21), even though the carotid arterial pressure was increased by the same magnitude observed during the norepinephrine-induced water diuresis (11).

In contrast to the results of carotid constriction above the sinus, the same degree of bilateral constriction below the carotid sinus (but well above the thyrocarotid junction) was found in the present study to be associated with an antidiuretic response which was similar to that observed during the i.v. infusion of ISO. The lowering of perfusion pressure to the carotid sinus was also associated with a substantial increase in systemic arterial pressure. Since this increase in arterial pressure could have initiated a reflex at the level of the aortic arch which might have suppressed rather than stimulated the release of vasopressin, these studies were performed in vagotomized animals to avoid such opposing parasympathetically mediated messages. Although the increase in renal vascular resistance which occurred in these experiments could have contributed to the observed antidiuresis, the increase in renal arterial pressure would have opposed this effect and by itself tended to blunt any antidiuretic response (22). Since these effects of carotid constriction below the carotid sinus on systemic arterial pressure and renal hemodynamics were probably sympathetically mediated, these results support our previous conclusion that the effect of alpha-adrenergic stimulation on renal water excretion is mediated primarily by suppression of vasopressin (11). More specifically, although systemic alpha-adrenergic stimulation probably predominated in the present studies

during carotid constriction below the carotid sinus and systemic beta-adrenergic stimulation predominated during the i.v. infusion of ISO, the effect on renal water excretion was the same in both instances. These similar effects on water excretion presumably resulted from the fact that the change in arterial pressure at the level of the carotid baroreceptors was the same in both circumstances. If an intrarenal effect on renal hemodynamics or an interaction with the action of vasopressin on renal tubular water permeability were the predominant pathway whereby alpha- and beta-adrenergic stimulation affect water excretion, then opposite rather than similar effects on water excretion would have been expected during the carotid constriction and i.v. ISO studies.

These present results thus suggest that the capacity of beta-adrenergic stimulation to increase vasopressin release was mediated primarily by the effect of i.v. ISO on systemic hemodynamics and baroreceptor neural tone. The previous observation that i.v. prostaglandin $E_1$, although not a beta-adrenergic agonist, produced similar effects as ISO on systemic hemodynamics, vasopressin release, and water excretion (23) is also compatible with this interpretation. More definitive studies were, however, necessary before concluding that the capacity of beta-adrenergic stimulation to increase vasopressin release was baroreceptor-mediated. In the present study neither cervical sham-operation nor bilateral cervical vagotomy abolished the antidiuretic effect of i.v. ISO. This antidiuretic effect of i.v. ISO, however, was abolished by a combination of bilateral cervical vagotomy and carotid sinus denervation. It thus seems that denervation of the cardiac, aortic arch, and carotid sinus baroreceptors is sufficient to abolish the capacity of beta-adrenergic stimulation to increase vasopressin release. Taken together, the findings in our present and previous studys (21) support the tentative hypothesis that many nonosmotic stimuli which alter vasopressin release may be mediated by changes in arterial baroreceptor tone. Further studies are, however, necessary in other situations that involve alterations in volume status, stress, and administration of other drugs. In this regard, we have recently found that nicotine does not directly stimulate the release of vasopressin but rather also seems to increase vasopressin release by altering baroreceptor neural tone (24).

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