The Antidiuretic Activity of 2-Amino-4-(4-chlorophenyl)-2-imidazoline (MJ 9465-2): Probable Release of Endogenous Antidiuretic Hormone (ADH) (37557)

DAVID DEITCHMAN AND ALLEN W. GOMOLL

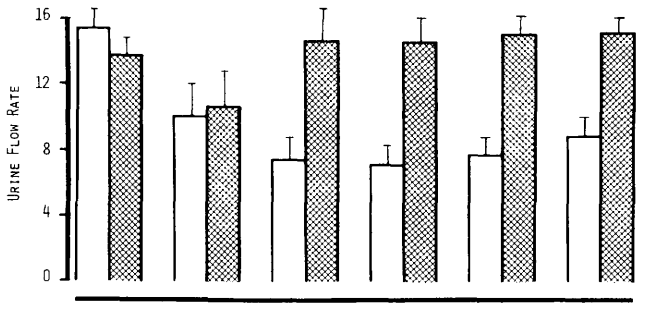
Department of Pharmacology, Mead Johnson Research Center, Evansville, Indiana 47721

MJ 9465-2 [2-amino-4-(4-chlorophenyl)-2-imidazoline HBr] one of a series of substituted 2-amino-4-phenyl-2-imidazoline derivatives, lowers systolic blood pressure in the DOCA-hypertensive rat (1). However, the rapid intravenous administration of MJ 9465-2 into normotensive, anesthetized dogs has previously been shown to enhance cardiovascular activity through at least two mechanisms, each of which produces an augmentation of blood pressure, myocardial contractile force and heart rate (2). The initial component of this response is dependent upon (a) functional sympathetic ganglia, (b) efferent pathways arising in the CNS, and (c) afferent fibers situated primarily in the vagus nerve. The second, slower phase of the response relies, in part, on the release of transmitter (norepinephrine) from sympathetic nerve terminals and/or chromaffin tissue. However, data from those experiments indicated that an additional factor, devoid of influence on contractile force and heart rate, was involved in this pressor response.

Further observations have indicated that these MJ 9465-2-elicited hemodynamic changes were maintained in nephrectomized dogs suggesting that activation of the renin–angiotensin system was not responsible for the recorded pressor responses. Consequently, another endogenous polypeptide with vasopressor properties, antidiuretic hormone (ADH), was considered. Various experimental challenges are known to release ADH from canine posterior pituitary gland. For example, (a) stimulation of the central end of the cut vagus nerve (3), hypothalamus (4) or midbrain (5), (b) carotid chemoreceptor excitation (6), and (c) bilateral carotid occlusion (7) elevate blood titers of the hormone. In the rat, administration of nicotine or lobeline (8) also causes liberation of ADH. It was reasoned, therefore, that if MJ 9465-2 evoked release of ADH, then urine volume excretion and solute concentration should decrease and increase, respectively, in animals with an established water diuresis. The present report describes experimental observations documenting these proposed urinary alterations and thereby provides evidence suggesting that ADH-release could contribute to the pressor response elicited by MJ 9465-2.

Method. A total of 48 fasted, mongrel dogs of either sex, weighing between 11 and 19 kg each were anesthetized with either chloralose (130 mg/kg) or sodium pentobarbital (30 mg/kg) administered iv. Chloralose was dissolved in polyethylene glycol (130 mg/ml) and diluted with saline prior to injection. Dogs were intubated and then ventilated mechanically (Harvard respirator) with room air at a rate of 16–17/min and a volume equivalent to 20 ml/kg. Mean arterial blood pressure (MABP) was monitored from a branch of the right femoral artery using a Statham pressure transducer (P23Dd). A branch of each femoral vein was cannulated for infusion of fluid and injection of drugs. In three dogs a catheter was advanced into the thoracic aorta via the left femoral artery for ia drug administration. Heart rate (HR) was monitored via a cardiotachometer. Hemodynamic measurements were recorded on a Beckman-Offner Type R dynograph. The ureters were exposed through a lower abdominal midline incision and cannulated near the bladder for bilateral collection of urine.

Either water or saline diuresis was established by infusion of a hypotonic (1.8%



OSMOLALITY	136	397	193	442	435	501	482	478	420	445	335	426
± SEM	13	48	16	48	38	70	54	56	45	46	40	45
MABP	131	132	178	189	145	150	140	141	138	137	137	134
± SEM	6	7	12	9	8	7	7	6	7	5	7	4

FIG. 1. Effects of MJ 9465-2 (80 μg/kg iv) on urine flow rate (ml/3 min), urine osmolality (mOsm/kg H_2O) and mean arterial blood pressure (mm Hg) as a function of time. Dogs were anesthetized with chloralose and hydrated with solutions containing either 1.8% glucose + 0.14% NaCl (open bar) or 0.9% NaCl (shaded bar). Vertical bars represent ± 1 SEM.

glucose + 0.14% NaCl) or an isotonic (0.9% NaCl) saline solution, respectively (3). Chloralose-anesthetized dogs were hydrated with an initial fluid load (30 ml/kg) of either hypotonic or isotonic solution infused iv at a rate of 15 ml/min. A second, or sustaining, infusion of identical composition, and to which gallamine[1] (0.027 mg/kg/min) and chloralase (0.5–1.0 mg/kg/min) were added, was continuously administered at a rate of 5–6 ml/min. Atropine SO_4 (0.5 mg/kg) and a priming dose of gallamine (0.4 mg/kg) were given to all animals when the sustaining infusion was initiated. Dogs anesthetized with pentobarbital received only a sustaining infusion (5–6 ml/min) of either hypotonic or isotonic solution following completion of surgery. One hour later, pentobarbital (4 mg/kg/min) and gallamine (0.027 mg/kg/min) were added to the infusate, and a priming dose of gallamine (0.4 mg/kg) was injected iv. Chlorisondamine[2] (5 mg/kg iv) was administered in four pentobarbital and five chloralose anesthetized dogs hydrated with hypotonic solution.

Following a variable interval of hydration (2–5 hr) to establish an adequate, steady-state diuresis (≧ 3 ml/min), 3-min urine samples were collected during a 15-min control period. Volumes were measured and the mean (ml/3 min) was utilized as basal urine flow. Subsequently, MJ 9465-2 (80 μg/kg, iv or ia) or ADH[3] (10 mU/kg, iv), was injected *rapidly*, and 3-min urine specimens were collected from the time of drug administration until urinary output returned to control levels. Urine osmolality (mOsm/kg H_2O) was determined on 2 ml aliquots of each sample by freezing point depression utilizing a Fiske osmometer (Model RAC 16).

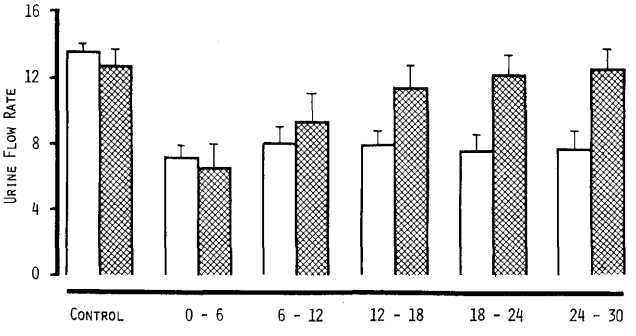
Results. MJ 9465-2. Steady-state urine flow, urine osmolality, mean arterial blood pressure, and the effects of the *rapid* iv injection of MJ 9465-2 on these variables were essentially independent of the type of anesthetic administered (Figs. 1 and 2, Table I). In contrast, MJ 9465-2-induced changes in urine osmolality and volume excretion were totally dependent on the tonicity of the solution used for hydration (Fig. 1).

In animals hydrated with the glucose–NaCl solution and excreting hypotonic urine, flow began to decline within the first 6 min after iv administration of MJ 9465-2 and then

[1] Gallamine triethiodide (Flaxedil) kindly supplied by Lederle Laboratories, Pearl River, NY 10965.

[2] Chlorisondamine Cl (Ecolid) kindly supplied by Ciba Pharmaceutical Co., Summit, NJ 07901.

[3] ADH (Pitressin) obtained from Parke-Davis, Detroit, MI 48232.



OSMOLALITY	124	457	238	505	491	609	507	566	492	517	453	485
± SEM	12	20	20	18	31	32	31	37	45	25	54	24
MABP	140	142	201	197	161	159	151	153	148	150	147	147
± SEM	9	7	9	11	10	7	9	6	9	7	9	7

FIG. 2. Effects of MJ 9465-2 (80 μg/kg iv) on urine flow rate (ml/3 min), urine osmolality (mOsm/kg H_2O) and mean arterial blood pressure (mm Hg) as a function of time. Dogs were anesthetized with pentobarbital and hydrated with solutions containing either 1.8% glucose + 0.14% NaCl (open bar) or 0.9% NaCl (shaded bar). Vertical bars represent ± 1 SEM.

continued to decrease over the next 18–24 min. Coincident with this reduced volume excretion, urine osmolality increased 200–300% above control values (Figs 1 and 2). Subsequently, urine flow and osmolality returned gradually to preinjection levels. In three dogs, no significant change in urine flow or osmolality was observed in response to ia injections of MJ 9465-2. In contrast, the typical antidiuretic response was still elicited by iv administration of MJ 9465-2 (Fig. 3).

Dogs hydrated with 0.9% NaCl and excreting slightly hypertonic urine also exhibited a similar reduction in urine volume excretion within the first 6 min following drug administration. However, in contrast to dogs undergoing water diuresis, urine osmolality increased only slightly (20%) and urine flow rate rapidly returned to control levels (Figs. 1 and 2).

Rapid iv injection of MJ 9465-2 elicited a marked and immediate pressor response, average 107–126 mm Hg (Table I), which returned to control levels within 3 to 12 min (Fig. 2). Thus, during the periods of maximum antidiuresis, MABP was elevated only slightly (5–10%). HR response was more variable (Table I). In general, tachycardia was demonstrable immediately following drug administration. This positive chronotropic response was succeeded by a decreased heart rate which gradually returned to or above control values during the course of the experiment. In several dogs, however, only a monophasic response was observed; either tachycardia or bradycardia. Rapid ia injections of MJ 9465-2 had no discernible effect on either BP or HR.

Chlorisondamine Pretreatment. Although mean arterial blood pressure and heart rate were lower in chlorisondamine-treated than in nontreated dogs, steady-state (control) urine flow and osmolality were comparable (Table I). The antidiuretic response elicited by iv injection of MJ 9465-2 in water-loaded, ganglion-blocked dogs was identical in profile to that obtained in the absence of chlorisondamine. Urine flow decreased approximately 50%, while osmolality increased 240% (Fig. 4). The immediate (15–20 sec) vasopressor and positive chronotropic responses that are dependent upon functional sympathetic ganglia were markedly attenuated in chlorisondamine-treated dogs, while the secondary, or latent, pressor component elicited by MJ 9465-2 was still operative. Slowing of heart rate was not observed (Table I).

Vasopressin (ADH). Volume excretion and urinary osmolality changes elicited by iv administration of 10 mU/kg of ADH in water-

TABLE I. Cardiovascular and Urine Osmolality Responses to MJ 9465-2 (80 μg/kg) or ADH (10 mU/kg) in Anesthetized Dogs Hydrated with Hypotonic or Isotonic Solutions.

Anesthetic agent	Drug treatment	N^a	Dog wt (kg)[b]	Mean arterial blood pressure (mm Hg)		Heart rate (bpm)			Urine osmolality (mOsm/kg H_2O)	
				Control	Maximum increase from control	Control	Maximum increase from control	Maximum decrease from control	Control	Peak response
				Hypotonic (1.8% glucose + 0.14% NaCl) hydration						
Chloralose	MJ 9465-2	10	12.7	131 ± 6[c]	107 ± 14	148 ± 8	53 ± 15	38 ± 10	136 ± 13	511 ± 52
	ADH	5	13.0	137 ± 7	49 ± 6	142 ± 17	—	30 ± 8	134 ± 19	437 ± 23
Pentobarbital	MJ 9465-2	7	13.4	140 ± 9	116 ± 6	164 ± 9	51 ± 13	28 ± 9	124 ± 12	534 ± 33
Chloralose or pentobarbital	Chlorisondamine[d] MJ 9465-2	9	14.2	107 ± 7	54 ± 7	115 ± 3	18 ± 6	—	143 ± 16	521 ± 49
				Isotonic (0.9% NaCl) hydration						
Chloralose	MJ 9465-2	6	12.8	132 ± 7	126 ± 11	145 ± 11	60 ± 13	44 ± 16	397 ± 48	517 ± 66
	ADH	4	12.5	135 ± 9	56 ± 6	147 ± 9	—	48 ± 11	392 ± 26	468 ± 22
Pentobarbital	MJ 9465-2	7	15.4	142 ± 7	123 ± 13	147 ± 13	58 ± 12	20 ± 7	457 ± 20	650 ± 47

[a] Number of observations.
[b] Average wt of *n* dogs.
[c] Mean ± SEM group.
[d] Chlorisondamine (5 mg/kg, iv) administered prior to establishing basal levels and challenge with MJ 9465-2.

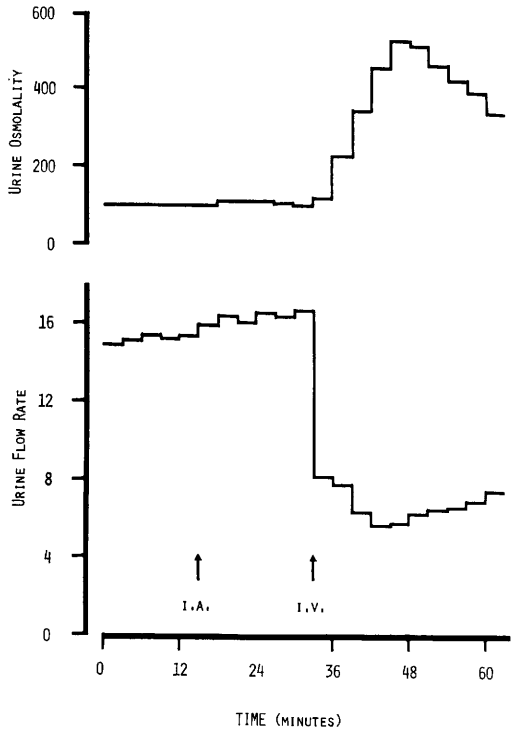
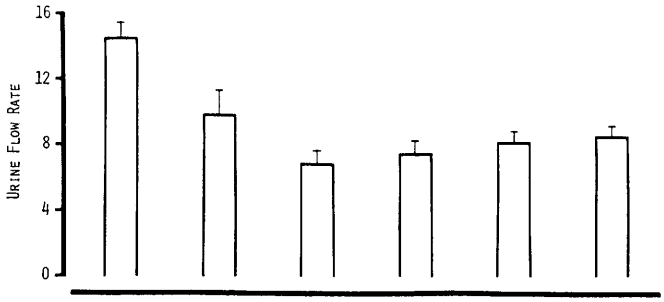


FIG. 3. Effects of ia and iv administration of MJ 9465-2 (80 μg/kg) on urine flow rate (ml/3 min) and urine osmolality (mOsm/kg H_2O) as a function of time. Dogs were anesthetized with pentobarbital and hydrated with a solution containing 1.8% glucose + 0.14% NaCl.

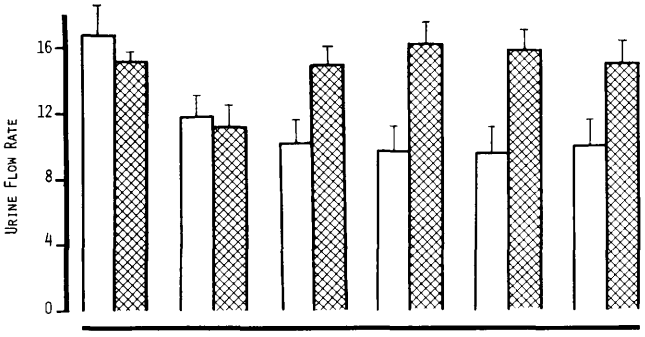
or saline-hydrated, chloralose-anesthetized dogs are summarized in Fig. 5. Dogs undergoing a saline diuresis responded to ADH injection with transient reductions in urine flow and only minimal increases (16%) in urine osmolality. In contrast, urinary volume excretion was reduced 43% and urine osmolality elevated 200% in dogs excreting a dilute urine. The time-course and magnitudes of fall in urine flow and elevation in urine osmolality following adminstraton of ADH were qualitatively similar to the profile recorded following injection of MJ 9465-2 (Fig. 1).

Discussion. Antidiuretic hormone (ADH) causes increased kidney reabsorption of fluid by enhancing the permeability of the distal tubule and collecting ducts to water. The amount of water reabsorbed, in the presence of ADH, depends upon (a) the solute concentration of fluid entering the distal tubule and collecting ducts, (b) the concentration gradient between tubular fluid and the interstitial spaces within the inner medulla of the kidney, and (c) the rate of urine flow. Lowered plasma ADH concentrations may result from a reduction in endogenous release of the neurohypophyseal hormone. One mechanism mediating inhibition is thought to involve activation of stretch receptors in the



OSMOLALITY	143	222	469	485	429	371
± SEM	16	19	51	47	32	31
MABP	107	150	139	131	127	124
± SEM	7	12	11	9	8	8

FIG. 4. Effects of MJ 9465-2 (80 μg/kg iv) on urine flow rate (ml/3 min), urine osmolality (mOsm/kg H_2O), and mean arterial blood pressure (mm Hg) as a function of time. Ganglionic transmission in these animals was blocked by administration of chlorisondamine (5 mg/kg iv). Dogs were anesthetized with either chloralose or pentobarbital and hydrated with a solution containing 1.8% glucose + 0.14% NaCl. Vertical bars represent ± 1 SEM.



	CONTROL		0 - 6		6 - 12		12 - 18		18 - 24		24 - 30	
					TIME (MINUTES)							
OSMOLALITY	134	392	229	429	385	458	413	430	408	412	378	400
± SEM	19	26	20	26	34	19	23	22	21	25	28	32
MABP	137	135	176	178	165	163	158	152	154	147	153	144
± SEM	7	10	10	10	8	7	7	7	7	7	7	7

FIG. 5. Effects of ADH (10 mU/kg iv) on urine flow rate (ml/3 min), urine osmolality (mOsm/kg H_2O) and mean arterial blood pressure (mm Hg) as a function of time. Dogs were anesthetized with chloralose and hydrated with solutions containing either 1.8% glucose + 0.14% NaCl (open bar) or 0.9% NaCl (shaded bar). Vertical bars represent ± 1 SEM.

wall of the left atrium as can be demonstrated by increasing blood volume (9–11) or mechanically stretching the atria (6).

In the present investigation, steady-state urine flows were of comparable magnitude under all experimental conditions. Plasma titers of ADH were probably depressed, if not abolished, in response to infusion of large volumes of isotonic or hypotonic solution (10). Under these conditions, urine osmolality should approximate solute concentration in fluid traversing the distal tubule and collecting duct. Thus, if plasma levels of ADH increase, dogs hydrated with 0.9% NaCl and excreting a slightly hypertonic urine, would have less free water available for reabsorption than dogs in which a water diuresis had been established. Moreover, the excretion profile in dogs undergoing a saline diuresis should reflect the influence of factors other than water reabsorption, such as drug-induced depression of renal blood flow and/or reduced glomerular filtration rate, on urinary output.

The results of these experiments demonstrate that exogenously administered ADH has no appreciable influence on volume excretion or urine osmolality in dogs hydrated with isotonic saline. In contrast, ADH produced a substantial decrease in urine flow concomitant with a marked elevation of urine osmolality in water-hydrated dogs. MJ 9465-2 has been shown to evoke a profile of urinary responses essentially similar to those of ADH under equivalent experimental conditions. However, MJ 9465-2 exhibited no direct ADH-like activity since ia administration of this compound at an effective iv dose, failed to alter the osmolality and/or volume excretion pattern. These observations thus lend support to a previous hypothesis (2) suggesting that rapid iv adminstration of MJ 9465-2 in the dog causes the release of an antidiuretic-like pressor material, probably vasopressin.

The data further indicate that drug-elicited hemodynamic changes have only a transient depressant effect on urinary excretory volume and total solute excretion. Finally, the demonstration of a typical pattern of MJ 9465-2-induced renal alterations in chlorisondamine-pretreated dogs indicates that this antidiuretic response, like the latent pressor component, is independent of a functional autonomic nervous system.

Summary. Injections of either MJ 9465-2 or ADH, iv, depressed and increased, respectively, urine volume excretion and osmolality in dogs undergoing water diuresis.

In contrast, only transient changes in urine flow and osmolality were observed in animals hydrated with saline and excreting an iso- or hypertonic urine. As demonstrated by ia administration, MJ 9465-2 (80 μg/kg) is devoid of any direct ADH-like activity. The antidiuretic response elicited by MJ 9465-2 was independent of the anesthetic agent administered. Ganglionic blockade, which abolished the acute pressor and positive chronotropic responses elicited by MJ 9465-2, had no effect on either the delayed pressor component or the induced antidiuretic activity. The data suggest that iv injection of MJ 9465-2 causes the release of a vasoactive, antidiuretic material, probably vasopressin.

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