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Effect of the gadolinium ion on body fluid regulation

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Abstract

Both osmoreception and baroreception are thought to involve ion channels that are sensitive to changes in membrane stretch. We investigated the effect of a blocker of stretch-activated ion channels, the Gd^{3+} ion, on osmoregulatory and cardiovascular responses in the intact rat. Intracerebroventricular injection of 50–100 nmol Gd^{3+} reduced thirst induced by various treatments. Similar doses also reduced intake of saline induced by various treatments. Intracerebroventricular injection of 100 nmol Gd^{3+} transiently increased arterial pressure and reduced the pressor response to intracerebroventricular angiotensin II (Ang II). Systemic administration of Gd^{3+} failed to alter thirst, except for a high dose (270 μmol/kg) that induced illness. This high dose failed to prevent urinary hypertonicity and excretion of a load of hypertonic NaCl. Intravenous infusion of 270 μmol/kg of Gd^{3+} reduced blood pressure and pressure responses to intravenous phenylephrine, but did not reduce the baroreceptor reflex control of heart rate. We conclude that the effects of Gd^{3+} on thirst and on the cardiovascular system are probably not due to a direct effect of the drug on stretch-sensitive ion channels. Instead, many of the effects of Gd^{3+} were compatible with blockade of voltage-gated Ca^{2+} channels.

Keywords: Stretch-sensitive ion channels; Gadolinium; Thirst; Sodium intake; Osmoreceptors; Baroreceptors

1. Introduction

Gilman (1937) concluded that changes in thirst that are seen after administration of hypertonic NaCl depend on the volume of the cells. Verney (1947) showed that the release of ADH similarly depends on cell volume, and coined the term *osmoreceptor* for the sensory elements that mediate these responses.

The mechanism by which these osmoreceptors transduce changes in cell volume is still not clear, but recent work suggests that stretch-sensitive ion channels are involved. In electrophysiological studies, it was shown that the resting potential of magnocellular neurons of the supraoptic nucleus depends on the tonicity of the medium (Oliet and Bourque, 1993a). The changes in resting potential are caused by changes in conductance to cations (Oliet and Bourque, 1993b). It is thought that cell shrinkage causes the opening of cation channels that are sensitive to stretch, allowing entry of sodium into the cell. The resulting depolarization of the membrane would promote the generation of action potentials, leading to the release of ADH. Conversely, overhydration of the cell would cause these channels to close, reducing the release of ADH (Bourque and Oliet, 1997). The current caused by the opening of these channels can be blocked with the gadolinium ion, Gd^{3+} (Oliet and Bourque, 1996), a drug commonly used to block stretch-sensitive ion channels in in vitro conditions (Hamill and McBride, 1996).

These in vitro studies indicate that Gd^{3+} may be an attractive tool to investigate the action of osmoreceptors in the intact animal. However, Gd^{3+} also blocks several voltage-gated Ca^{2+} channels (Hamill and McBride, 1996). In addition, it binds tightly to anions present in the body fluids (Caldwell et al., 1998). To explore the effectiveness of Gd^{3+} as an osmoreceptor blocker, we tested the effect of this drug on thirst induced by various stimuli, including water deprivation, injection of angiotensin II (Ang II) in the lateral brain ventricle, intragastric injection of hypertonic NaCl and injection of the diuretic furosemide. We also measured the effect of Gd^{3+} on sodium intake. Since ions such as Gd^{3+} do not easily cross the blood–brain barrier, and osmoreceptors are present both inside and outside this barrier (McKinley et al., 1978), we measured the effects of Gd^{3+}

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administered in the lateral brain ventricle as well as in the circulation.

Thirst and sodium intake also depend on blood pressure and blood volume. To test whether drug-induced changes in fluid intake could be secondary to changes in blood pressure, we measured the effect of gadolinium on blood pressure. In addition, the sensory mechanism involved in baroreceptor reflexes may consist of stretch-sensitive ion channels that are sensitive to Gd^{3+} (Sullivan et al., 1997). To explore the effectiveness of Gd^{3+} as a baroreceptor blocker, we measured the effect of intravenous administration of Gd^{3+} on heart rate changes induced by changes in blood pressure.

2. Methods

2.1. Animals

To study effects of intracerebroventricular administration of Gd^{3+}, we used male Holtzman rats fed with Purina rodent pellets (Purina, Campinas, SP, Brazil). To study effects of systemic administration, we used male Wistar rats fed with Nuvilab pellets (CR1, Nuvital, Colombo, PR, Brazil). Rats were housed individually and kept in a temperature-controlled room on a 12:12 light/dark cycle beginning at 0700 h. Food and tap water were available ad libitum unless otherwise noted. All experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Brain cannulas and injections

Under ketamine anesthesia (120 mg/kg ip), a stainless-steel guide cannula (12 mm long, 0.7-mm OD) was implanted in the brain, with its opening protruding into the top of the lateral ventricle (0.3 mm behind bregma; 3.7 mm from the skull surface and 1.6 mm from sagittal midline; incisor bar at the level of the interaural line). The cannula was anchored with dental cement to two stainless steel screws inserted in the skull. A close fitting stylet was inserted in the cannula to keep the lumen free of debris and clots. Rats received penicillin (30,000 IU im) presurgically and were allowed to recover from surgery for at least 4 days.

To make intracerebroventricular injections, the rat was removed from the cage and the stylet was replaced by an injector that protruded 2.0 mm beyond the tip of the guide cannula. The injector was connected by PE-10 tubing to a hand-held 10-μl Hamilton syringe. All injections were 1.0 μl/30 s. After the injection, the injector was removed, replaced by the stylet, and the rat was returned to its cage.

On completion of the experiments, rats were deeply anesthetized (1.5 ml of 10% chloral hydrate, ip) and perfused with 10% formalin through the left ventricle of the heart. The frozen brains were cut in 50-μm coronal sections and stained with hematoxylin–eosin. Only animals with injections into the lateral ventricle were considered for analysis.

2.3. Arterial cannulas

Arterial cannulas were made from polyethylene tubing (PE-10 joined to PE-50). Under halothane (2% in 100% oxygen) or tribromoethanol anesthesia (20 mg/100 g body weight, ip), the cannula was inserted into the femoral artery and advanced until the cannula tip was in the abdominal aorta, below the renal artery. The other end was tunneled subcutaneously and exteriorized at the nape of the neck. All rats had full use of the operated leg.

2.4. Carotid cannulas and carotid infusions

Under halothane anesthesia, silicone rubber cannulas were inserted in both external carotid arteries and advanced to bring the cannula tip to the junction of internal and external carotid arteries (Schoorlemmer et al., 2000). This procedure blocks flow through the external but not the internal carotid arteries. All fluid infused into the cannula enters the internal carotid artery, which supplies blood to much of the brain. Rats were allowed to recover from surgery for at least 10 days.

To estimate the concentration of the drugs infused in the blood entering the brain, we measured blood flow through the cannulated carotid arteries, using a transit time flow meter and probe (T206 and 1RB, respectively, Transonic Systems, Ithaca, NY, USA). In four rats (body weight, 428 ± 13 g) anesthetized with thiopental (40 mg/kg iv) about 3 weeks after implantation of the carotid cannulas, blood flow was 3.9 ± 0.5 ml/min on each side.

To infuse, the cannulas were connected with polyethylene tubing to 'airtight' Hamilton syringes, mounted on a syringe pump (BSP1000, Braintree Scientific, USA). Solutions infused into the carotid artery contained, besides 0.3 M NaCl and a varying concentration of $GdCl_3$, a small amount of heparin (1 IU/ml).

2.5. Venous cannulas and infusions

Venous cannulas were made from silicone rubber (1.0-mm OD) joined to polyurethane (1.0-mm OD). Under halothane anesthesia, the silicone end was inserted in the femoral vein and advanced 4 cm to bring the tip in the abdominal vena cava. The polyurethane end was tunneled under the skin and exteriorized on the back between the shoulder blades. Rats were allowed to recover from surgery for at least 10 days.

To infuse, the cannulas were connected to 'airtight' Hamilton syringes, mounted on a syringe pump (BSP1000, Braintree Scientific).

2.6. Drugs

To study the effects of central administration of Gd^{3+}, $GdCl_3$ hexahydrate (from Aldrich) was dissolved in 0.9%

NaCl. To study the effect of systemic administration, $GdCl_3$ was dissolved in water containing various concentrations of NaCl, as described in the experimental protocols. Furosemide (from Sigma) and Ang II (Sigma) were dissolved in 0.9% saline.

2.7. Statistics

Data are expressed as means ± standard error of the mean. Data were analyzed by two-way analysis of variance, with time as a repeated measure. To locate significant differences, we used the Student–Newman–Keuls post hoc test for comparisons between treatments, and Dunnett's test for comparisons with pre-infusion data in the same rats. Differences were considered significant if the chance of their occurrence by accident was less than 0.05.

2.8. Experimental protocols

Rats received several doses of Gd^{3+} in a counterbalanced design. The interval between experiments was 3 days unless stated otherwise.

2.9. Thirst after injection of Gd^{3+} in the lateral brain ventricle

2.9.1. Thirst induced by water deprivation

Rats (280–320 g) were deprived of water, but not food, for 24 h. $GdCl_3$ (0, 50 or 100 nmol in 1-μl 0.15 M NaCl) was injected intracerebroventricularly, and water became available 20 min later. Water intake was recorded at 15, 30 and 60 min. Food was not available during the drinking period.

2.9.2. Thirst induced by hypertonic saline

Rats (280–320 g) received an intragastric gavage of 2 ml of 12% NaCl, and food and water were removed. $GdCl_3$ (0, 50 or 100 nmol in 1-μl 0.15 M NaCl) was injected intracerebroventricularly 40 min later. Another 20 min later, water was returned. Water intake was recorded at 15, 30 and 60 min.

2.9.3. Thirst induced by furosemide

Rats (280–320 g) received an injection of the diuretic furosemide (10 mg/rat sc), and food and water were removed. $GdCl_3$ (0, 25, 50, 100 or 200 nmol in 1-μl 0.15 M NaCl) was injected intracerebroventricularly 40 min later. Another 20 min later, water was returned. Water intake was recorded at 15, 30 and 60 min.

2.9.4. Thirst induced by Ang II

Rats (280–320 g) received an intracerebroventricular injection of $GdCl_3$ (0, 50 or 100 nmol in 1-μl 0.15 M NaCl), followed 20 min later by an intracerebroventricular injection of Ang II (50 ng in 1-μl 0.15 M NaCl). Cumulative water intake was recorded 15, 30 and 60 min after injection of Ang II. Food was not available during this test.

2.10. Sodium intake after injection of Gd^{3+} in the lateral brain ventricle

Rats used to measure the effect of $GdCl_3$ on sodium intake were allowed access to sodium solution for several days before the start of the experiment.

2.10.1. Sodium intake induced by Ang II

Rats (280–320 g) received an intracerebroventricular injection of $GdCl_3$ (0, 50 or 100 nmol in 1-μl 0.15 M NaCl), followed 20 min later by an intracerebroventricular injection of Ang II (50 ng in 1-μl 0.15 M NaCl). Intakes of water and 0.15 M NaCl were recorded 15, 30, 45 and 60 min after injection of Ang II. Food was not available during this test.

2.10.2. Sodium intake induced by furosemide

Rats (280–320 g) received an injection of furosemide (10 mg/rat sc) and were transferred to clean cages, with access to sodium-deficient food (powdered corn meal, 0.001% sodium, 0.33% potassium) and water, but not saline. The following day (24 h after the injection of furosemide), food was removed and 0.3 M NaCl was returned. $GdCl_3$ (0, 50, 100 or 200 nmol in 1-μl 0.15 M NaCl) was injected intracerebroventricularly 20 min before return of saline. Intakes of 0.3 M NaCl and water were measured at 15, 30, 60 and 120 min.

2.10.3. Sodium intake induced by water deprivation

Water deprivation not only induces a water deficit, but also a negative sodium balance (Schoorlemmer and Evered, 1993). We allowed fluid-deprived (24 h) rats (280–320 g) to drink water for 2 h to allow restoration of the water deficit before we gave them access to both 0.3 M NaCl and water (Sato et al., 1996). We recorded intake of water during the 2 h with access exclusively to water, and intakes of water and 0.3 M NaCl at 15, 30, 60 and 120 min after returning saline. $GdCl_3$ (0 or 100 nmol in 1-μl 0.15 M NaCl) was injected intracerebroventricularly 20 min before access to NaCl.

2.11. Effect of injection of Gd^{3+} in the lateral brain ventricle on arterial blood pressure

Rats (280–320 g) were fitted with an arterial cannula and were used to measure blood pressure and heart rate responses to intracerebroventricular Gd^{3+} the next day. After recording of resting values (polygraph, Narcotrace 40, Narco Bio-System, USA), an injector was inserted in the guide cannula and either the vehicle (1 μl of 150 mM NaCl) or $GdCl_3$ (100 nmol in 1-μl 150 mM NaCl) was injected. About 13 min later, an injector containing Ang II was inserted in the guide cannula, and angiotensin (50 ng in 1 μl) was injected 2 min later.

2.12. Thirst after systemic infusion of Gd^{3+}

2.12.1. Thirst induced by water deprivation

Four rats (297 ± 27 g) with venous cannulas were deprived of water, but not food, for 18 h. At the end of this period, food was removed, an intravenous infusion (0.1 ml/min/kg body weight) containing Gd^{3+} (0, 30 or 200 μmol/kg body weight, or 0-, 10- or 70-mg $GdCl_3$ hexahydrate/kg) was started and drinking water was returned. Ten minutes later, the infusion was stopped. We recorded latency to drink and intake of water at 5, 10, 20, 30 and 60 min. Treatments were administered in mixed order, at least 2 days apart.

2.12.2. Thirst and sodium excretion induced by intravenous infusion of hypertonic NaCl

Six rats (374 ± 7 g) with venous cannulas received an intravenous infusion of hypertonic NaCl (1.5 ml of 1.2 M NaCl in 10 min). The infusion fluid also contained Gd^{3+} (0, 8, 27, 80 or 270 μmol/kg body weight, or 0-, 3-, 10-, 30- or 100-mg $GdCl_3$ hexahydrate/kg). We recorded latency to drink, water intake at 5, 10, 20, 30 and 60 min, and the amount of water, sodium and potassium spontaneously voided during the drinking period. Treatments were given in random order, 2 days apart. However, the highest dose of Gd^{3+} caused a conditioned aversion to the infusion of hypertonic saline; rats failed to drink in subsequent experiments in which they received hypertonic NaCl, but no gadolinium. Therefore, rats were not used again after they had received this dose.

2.12.3. Thirst induced by intracarotid infusion of hypertonic NaCl

We used eight rats (446 ± 8 g) with cannulas in the femoral vein and both carotid arteries. Rats received a bilateral intracarotid infusion of 0.3 M NaCl (100 μl/min each side for 20 min) and a simultaneous infusion of 0.15 M NaCl intravenously (20 μl/min for 20 min). Gd^{3+} (13 or 27 μmol/kg, or 5- or 10-mg $GdCl_3$ hexahydrate/kg body weight) was added to either the intravenous or the intracarotid infusion. We recorded latency to drink and water intake during the 20-min infusion period. All rats received both treatments in random order, 1 week apart. In a separate experiment in these rats, water intake during an identical intracarotid infusion of 0.3 M NaCl in the absence of $GdCl_3$ was 5.4 ± 1.3 ml/20 min.

2.13. Cardiovascular effects of intravenous infusion of Gd^{3+}

We used rats (473 ± 14 g) with cannulas in the femoral artery and both femoral veins. Experiments started 1 week after implantation of cannulas. $GdCl_3$ (0, 27, 80, 135 or 270 μmol/kg body weight in 10 min in a volume of 1 ml/kg) was infused intravenously. Rats received several doses in mixed order, separated by at least 3 days. Because responses to the three highest doses were similar, they were combined for data analysis (80 μmol/kg, $n = 6$; 135 μg/kg, $n = 2$; 270 μg/kg, $n = 1$). Arterial pressure and heart rate were recorded for 70 min, starting 10 min before the beginning of the Gd^{3+} infusion, on a computer with an AD converter (PowerLab 4SP, AD Instruments, USA). Responses to intravenous administration of phenylephrine (1 and 2 μg/15 s) and sodium nitroprusside (5 μg/5 s) were measured before and after Gd^{3+} infusion, as well as during the second half of the Gd^{3+} infusion. To calculate baroreceptor sensitivity, we selected the time of the peak change in heart rate, and divided the change in heart rate (compared to the pre-injection value) by the change in blood pressure.

3. Results

3.1. Thirst after intracerebroventricular injection of Gd^{3+}

Intracerebroventricular injection of 50–100 nmol Gd^{3+} reduced thirst, whether it was induced by water deprivation, intragastric administration of hypertonic NaCl, treatment with the diuretic furosemide or intracerebroventricular injection of Ang II (Fig. 1). Higher doses usually had a stronger effect, but we did not always find a perfect dose–effect relationship (Fig. 1C and D).

3.2. Sodium intake after intracerebroventricular injection of Gd^{3+}

Doses of Gd^{3+} that reduced thirst also reduced sodium intake caused by intracerebroventricular injection of Ang II and by water deprivation, followed by 2 h of access to water (Fig. 2). Gd^{3+} also tended to reduce sodium intake caused by furosemide, but this type of salt intake seemed more resistant to Gd^{3+} than the others, since even very high doses of Gd^{3+} failed to significantly reduce it.

3.3. Other effects of intracerebroventricular injection of Gd^{3+}

In several rats, intracerebroventricular injection of 200 nmol induced salivation and tremors that started immediately after the injection and lasted about 30 s. Data from these experiments were not included in the analysis. In addition, four rats looked sick during the days after injection of this high dose, with weight loss and a rough fur. Data from these experiments were not included in the analysis. These animals were not used again.

3.4. Effect of intracerebroventricular Gd^{3+} on arterial pressure

Blood pressure increased for 30 s after injection of Gd^{3+} in the lateral ventricle, and fell back to baseline level within 5 min (Fig. 3, pressure increased 7 ± 3 mm Hg after Gd^{3+}, fell 1 ± 2 mm Hg in controls, $P < .05$, $n = 10$ and 7,

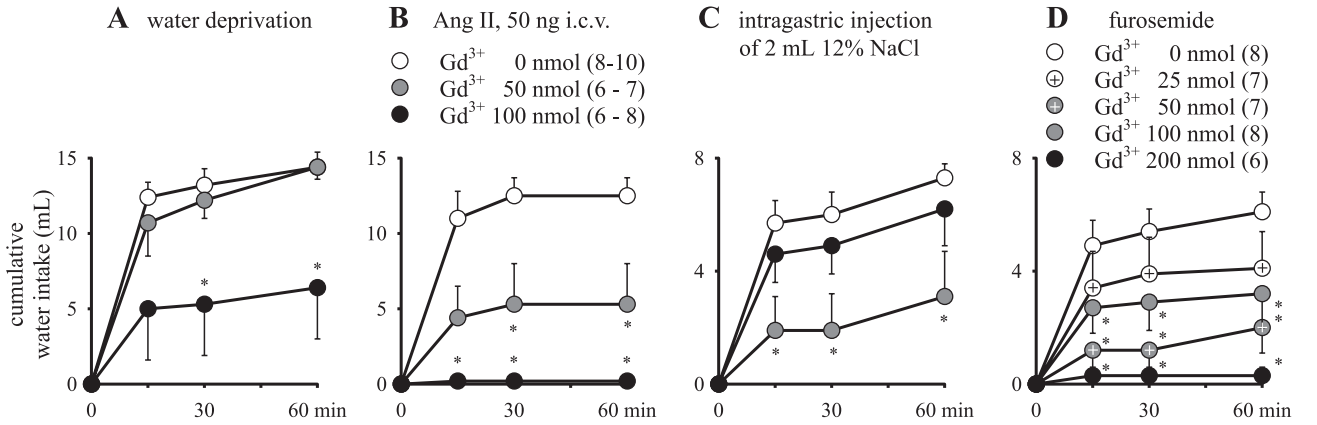

Fig. 1. Effect of injection of $GdCl_3$ into the lateral brain ventricle on water intake caused by (A) 24 h of water deprivation, (B) injection of Ang II in the lateral brain ventricle (50 ng at $t=0$), (C) intragastric injection of hypertonic NaCl (2 ml, 12% at $t=-60$ min), (D) removal of ambient sodium + injection of furosemide (10 mg/kg sc at $t=-24$ h). In all four tests, $GdCl_3$ was injected into the lateral brain ventricle at $t=-20$ min. Values are mean ± S.E. Numbers between brackets indicate number of rats. * Indicates difference from vehicle ($P<.05$).

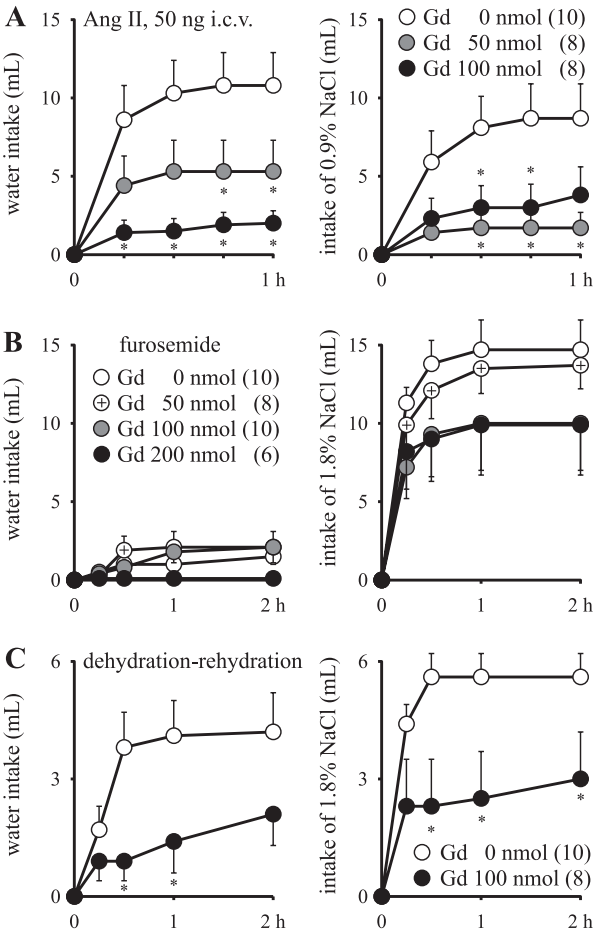

Fig. 2. Effect of injection of $GdCl_3$ into the lateral brain ventricle on intake of water and saline (NaCl solution) in a two-bottle test. (A) Intakes after injection of Ang II into the lateral brain ventricle (50 ng at $t=0$). (B) Intakes after removal of ambient sodium + injection of furosemide (10 mg/kg sc at $t=-24$ h). (C) Intakes after 24 h of water deprivation followed by 2 h of access to water. In all three tests, $GdCl_3$ was injected into the lateral brain ventricle at $t=-20$ min. Values are mean ± S.E. Numbers between brackets indicate number of rats. * Indicates difference from vehicle ($P<.05$).

respectively). Neither Gd^{3+} nor vehicle caused significant changes in heart rate (– 10 ± 5 vs. – 24 ± 13 bpm, respectively). Gd^{3+} reduced the pressor response to intracerebroventricular injection of 50 ng Ang II from 16 ± 2 to 7 ± 3 mm Hg ($P<.05$). Ang II did not alter heart rate, whether preceded by Gd^{3+} or vehicle (– 11 ± 7 vs. 1 ± 9 bpm).

3.5. Thirst and sodium excretion after systemic infusion of Gd^{3+}

Gd^{3+} did not affect drinking latency or water intake after water deprivation (Fig. 4A). Data from one trial in which a rat receiving the high dose became sick were discarded.

Intravenous infusion of hypertonic NaCl usually induced thirst within 5 min of the beginning of the infusion (range: 2 min and 45 s to 5 min and 30 s). Water intake was reduced when hypertonic NaCl was infused together with a high dose of $GdCl_3$ (Fig. 4B). This dose did not alter the latency to drink, but rats stopped drinking soon after they started, and did not resume drinking until about 25 min after the infusions had ended. Gadolinium did not seem to interfere with the act of drinking itself, since the rate of drinking during drinking bouts seemed normal. This high dose of $GdCl_3$ however caused a conditioned aversion to the infu-

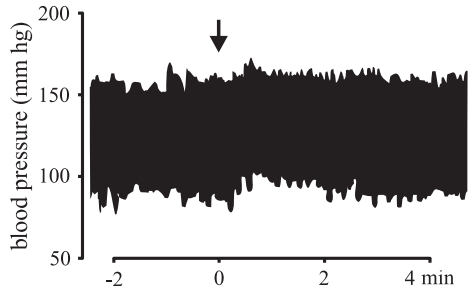

Fig. 3. Effect of injection of 100 nmol $GdCl_3$ (arrow) into the lateral brain ventricle on pulsatile arterial pressure in a single rat.

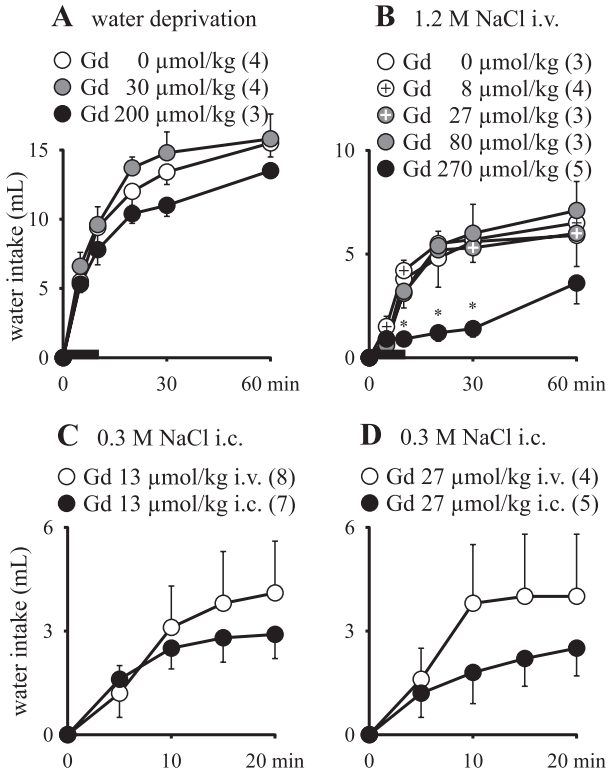

Fig. 4. Effect of systemic administration of $GdCl_3$ on thirst. (A) Effect of intravenous infusion of $GdCl_3$ (from 0 to 10 min, black bar) on thirst caused by 24 h of water deprivation. (B) Effect of intravenous infusion of $GdCl_3$ (from 0 to 10 min) on thirst induced by infusion of 1.2 M NaCl (1.5 ml, infused from 0 to 10 min). (C and D) Comparison of the effect of intravenous and intracarotid infusion of $GdCl_3$ (infused from $t=0$ to 20 min) on thirst induced by intracarotid infusion of 0.3 M NaCl (100 µl/min each side from $t=0$ to 20 min). Values are means ± S.E. Numbers between brackets indicate number of rats. * Indicates difference from vehicle ($P<.05$).

sion of hypertonic saline. This dose reduced body weight the next day by 17 ± 4 g. Gd^{3+} did not alter excretion of water, sodium or potassium (Table 1). During the 1-h drinking period, rats receiving the vehicle excreted 57 ± 9% of the infused sodium, and rats receiving the highest dose excreted 47 ± 6% of the sodium load infused.

Table 1
Effect of intravenous infusion of $GdCl_3$ on excretion of an intravenous load of hypertonic saline

$GdCl_3$ (µmol/kg)	*n*	Urine volume (ml/h)	Na^+ excretion (mmol/h)	K^+ excretion (mmol/h)
0	4	4.7 ± 0.7	1.0 ± 0.1	0.4 ± 0.1
8	4	4.2 ± 0.6	0.9 ± 0.1	0.4 ± 0.1
27	4	4.5 ± 0.5	0.9 ± 0.1	0.4 ± 0.1
80	3	3.8 ± 0.4	0.7 ± 0.0	0.3 ± 0.0
270	5	3.8 ± 0.4	0.9 ± 0.1	0.3 ± 0.0

Hypertonic NaCl (1.2 M, 1.5 ml) containing various amounts of $GdCl_3$ was infused intravenously in 10 min. Urine was collected for 1 h, starting at the beginning of the infusion period. Values are mean ± S.E. Differences between different doses of Gd^{3+} were not statistically significant (one-way ANOVA).

Intracarotid infusion of hypertonic NaCl reliably caused thirst, and Gd^{3+} did not influence either latency to drink or the volume drunk during the infusion period (Fig. 4C and D). Intracarotid infusion of the high dose of $GdCl_3$ was not tolerated well. Rats sometimes seemed confused during the second half of the infusion period and lost weight during the subsequent 24 h (26 ± 6 g vs. 5 ± 2 g after intravenous infusion of $GdCl_3$).

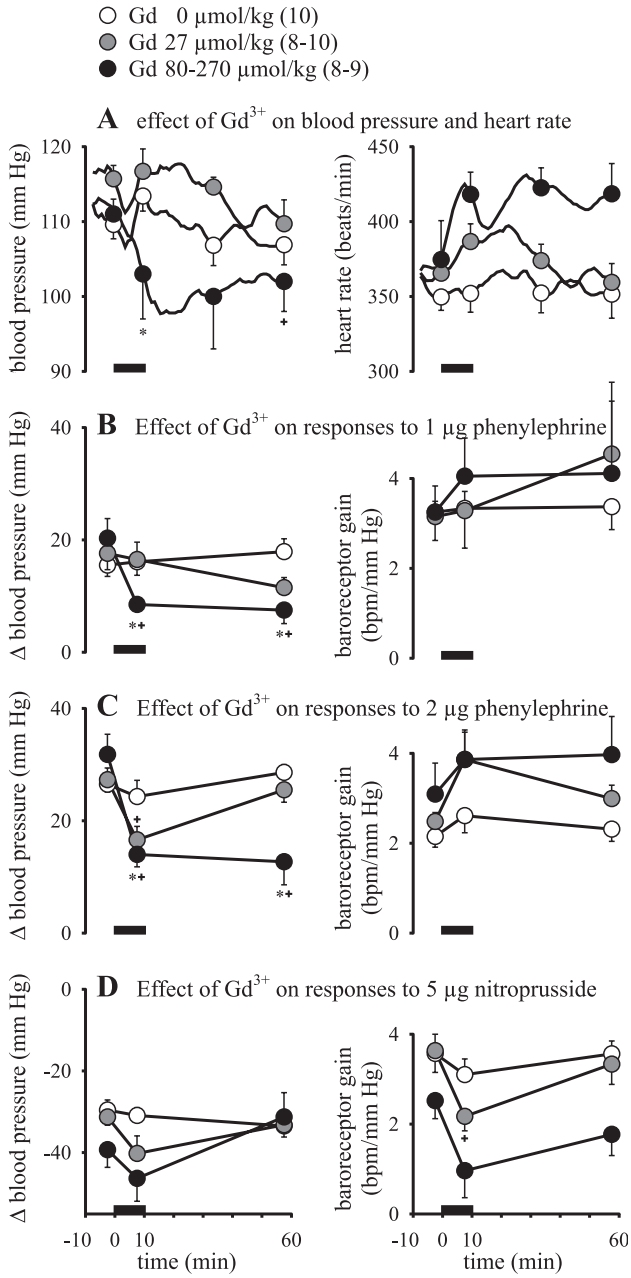

Fig. 5. Effect of intravenous infusion of $GdCl_3$ (from $t=0$ to $t=10$ min, black bar) on blood pressure, heart rate and responses to phenylephrine and sodium nitroprusside. Values are means ± S.E. Numbers between brackets indicate number of rats. * Indicates that the change (from $t=0$) induced by Gd^{3+} differs from the change induced by the control treatment ($P<.05$). + Indicates difference from pre-infusion value in the same rats ($P<.05$).

3.6. Cardiovascular effects of intravenous infusion of Gd^{3+}

Large doses of Gd^{3+} reduced blood pressure and tended to increase heart rate (Fig. 5A), but the effect was rather variable. Both rats that received 135 μg/kg of Gd^{3+} died in the night following the experiment. Gd^{3+} reduced pressor responses to phenylephrine but did not alter the sensitivity of the baroreceptor reflex to increases in blood pressure (Fig. 5B and C). The sensitivity of the baroreflex to a fall in blood pressure was reduced in rats treated with Gd^{3+}, but the effect was rather variable, and the changes in sensitivity induced by infusions of Gd^{3+} did not differ significantly from those induced by vehicle (Fig. 5D, $P>.2$).

4. Discussion

4.1. Effects of intracerebroventricular administration of Gd^{3+}

Injection of gadolinium into the lateral brain ventricle reduced thirst caused by hypertonicity of the body (water deprivation, intragastric load of hypertonic NaCl). In vitro studies have shown that Gd^{3+} blocks neurophysiological responses induced by hypertonicity (Oliet and Bourque, 1996), and our finding that gadolinium (50–100 nmol) blunts drinking responses induced by hypertonicity is consistent with this finding. Similar doses of Gd^{3+} also inhibited drinking induced by nonosmotic stimuli (sodium depletion, intracerebroventricular injection of Ang II). The inhibition by Gd^{3+} of drinking caused by nonosmotic stimuli is compatible with the idea that Gd^{3+} is acting as an osmoreceptor blocker, because drinking stimuli are additive. In addition, recent data indicate that peptides, including ANG II, may act by modifying the transport of ions through stretch-sensitive ion channels (Chakfe and Bourque, 2001). Thus, the high sensitivity to Gd^{3+} of thirst induced by Ang II could be due to a combination of removal of osmoreceptor input and blockade of the effect of Ang II on the osmoreceptors.

On the other hand, the inhibition of sodium intake caused by intracerebroventricular injection of Gd^{3+} is not due to a direct action of the drug on the osmoreceptors, since changes in tonicity of the cerebrospinal fluid do not seem to alter sodium intake in the rat (Denton et al., 1984; Epstein et al., 1984). In sheep, sodium intake depends on sodium concentration, rather than osmolality of the cerebrospinal fluid (Weisinger et al., 1982). It has been suggested that stretch-sensitive ion channels may mediate responses to both changes in cell volume and sodium concentration, because the permeability of these channels depends on the sodium concentration of the extracellular fluid (Voisin et al., 1999). In sheep, then, blockade of stretch-sensitive ion channels would presumably stimulate sodium appetite.

The mechanism of the reduced saline intake is not clear, but an action of Gd^{3+} on Ca^{2+} channels may be involved. Gd^{3+} is known to block voltage-gated Ca^{2+} channels (Boland et al., 1991; Elinder and Arhem, 1994; Hamill and McBride, 1996; Mlinar and Enyeart, 1993; De Castro e Silva et al., 1996). Administration of diltiazem, a blocker of L-type Ca^{2+} channels, in the lateral ventricle of the rat reduces salt appetite induced by sodium depletion (De Luca et al., 2002).

Since doses of Gd^{3+} that reduced sodium intake also reduced thirst, it seems likely that the inhibition of thirst was not due to osmoreceptor blockade, but to a more general impairment of fluid intake. We do not know at which level this impairment of drinking occurs, but the drug does not appear to seriously impair the motor act of drinking, because a high intracerebroventricular dose of Gd^{3+} did not block intake of saline in rats treated with furosemide. Reduced thirst, like reduced saline intake, could be due to blockade of Ca^{2+} channels, since thirst induced by intracerebroventricular injection of Ang II depends on central L-type Ca^{2+} channels (Calcagnetti and Schechter, 1993; Zhu and Herbert, 1997).

Injection of Gd^{3+} into the lateral brain ventricle increased blood pressure. It is unlikely that the effect of Gd^{3+} on blood pressure is due to blockade of osmoreceptors because reductions in the tonicity of the cerebrospinal fluid can reduce blood pressure (Chen, 1996). In addition, it is unlikely that the increase in blood pressure caused by intracerebroventricular Gd^{3+} is due to a direct effect of the drug on Ca^{2+} channels, because intracerebroventricular administration of Ca^{2+} channel blockers induces hypotension (Huang and Leenen, 1999) rather than hypertension.

4.2. Effects of systemic administration of Gd^{3+}

The highest dose of Gd^{3+} (270 μmol/kg iv) would increase Gd^{3+} concentration in the blood to over 1 mM, assuming the drug spreads evenly throughout the extracellular fluid. This is well above the 10–100 μM used to block these channels in vitro (Hamill and McBride, 1996), but since the drug chelates in the body fluids (Caldwell et al., 1998), the concentration of the free drug is probably very low. The reduction in water intake caused by this infusion is compatible with the idea that gadolinium acts on osmoreceptors to induce responses characteristic of hypotonicity. However, we failed to see any other signs compatible with blockade of osmoreceptors: urine remained concentrated after administration of Gd^{3+}, and the drug did not alter the excretion of the hypertonic load infused intravenously. The inhibition of drinking by intravenous Gd^{3+} may be due to illness rather than osmoreceptor blockade, as the dose that reduced water intake induced a conditioned aversion: subsequent hypertonic infusions failed to induce drinking, even in the absence of Gd^{3+}.

Intracarotid infusion of Gd^{3+} allows little time for the drug to chelate before reaching the osmoreceptors. Assum-

ing a carotid blood flow of 4 ml/side (see methods), and complete mixing of Gd^{3+} and blood, we estimate that intracarotid infusion of the high dose of Gd^{3+} would increase the Gd^{3+} concentration in the carotid arteries 0.35 mM above that of blood in the rest of the body. Intracarotid infusions affected cephalic targets, because intracarotid, but not intravenous infusions, caused a large weight loss. However, intracarotid infusion of Gd^{3+} did not significantly reduce water intake, suggesting that signals from extracephalic sites may contribute to the inhibition of thirst after intravenous infusion of high doses of Gd^{3+}.

Intravenous administration of Gd^{3+} did not affect the sensitivity of the baroreceptor reflex control of heart rate, but it reduced pressure and tended to increase heart rate, suggesting vascular resistance fell. Because blockade of Ca^{2+} channels causes vasodilation, the fall in blood pressure could be due to an action of Gd^{3+} on Ca^{2+} channels. In addition, pressor responses to phenylephrine depend on flow of Ca^{2+} into vascular smooth muscle cells, and the pressor responses to phenylephrine can be reduced by intravenous administration of Ca^{2+} channel blockers (Timmermans et al., 1983).

In conclusion, administration of Gd^{3+}, whether systemically or directly into the brain, alters responses that are under control of osmoreceptors. However, we doubt that these effects are due to a direct action of the drug on the osmoreceptors. With intracerebroventricular administration of Gd^{3+}, antidipsogenic doses also reduced saline intake, and since the reduced saline intake was not due to a direct effect of Gd^{3+} on the osmoreceptors, it seems likely that reduced thirst was not either. With systemic administration, the effect of the drug on thirst may depend on extracephalic receptors, and the drug failed to prevent concentration of urine and excretion of hypertonic NaCl. Therefore, Gd^{3+} is not an effective osmoreceptor blocker when it is administered in vivo. Gd^{3+}, administered into the circulation, is not an effective baroreceptor blocker either: although it had cardiovascular effects, it failed to reduce the sensitivity of the baroreceptor reflex control of heart rate.

Why the drug fails to block osmoreceptors is not clear. It is possible that the role of stretch-sensitive ion channels in the responses we are measuring is limited, since in the subfornical organ, which is a possible osmoreceptor site, the mechanism linking cell volume and resting potential of the neuronal membrane does not involve the cation channels described by Bourque and Oliet (Anderson et al., 2000). Alternatively, binding of the drug to anions in the blood (Caldwell et al., 1998) may prevent the action on stretch-sensitive ion channels.

A complete understanding of the properties of osmoreceptors and baroreceptors is a crucial missing step. Drugs that affect stretch-sensitive ion channels remain attractive candidates to selectively and directly alter the functioning of these sensors. However, the gadolinium ion is not an attractive candidate for the in vivo study of osmoreceptor and baroreceptor mechanisms.

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