Differential effects of aging on fluid intake in response to hypovolemia, hypertonicity, and hormonal stimuli in Munich Wistar rats


*Howard Florey Institute of Experimental Physiology and Medicine and †Department of Physiology, University of Melbourne, Victoria 3010, Australia; and ‡Baker Medical Research Institute, Prahran, Victoria 3181, Australia

Contributed by D. A. Denton, December 22, 2005

A significant proportion of aged humans may have impaired thirst and inadequate fluid intake after a period of fluid deprivation. We have studied the water drinking responses, relative to body weight, of Munich Wistar (MW) rats in response to osmotic, hypovolemic, dehydrational, and angiotensin (Ang)-related stimuli as they aged from 3 to 24 months. Young 3-months-old (m.o.) rats had the largest daily fluid intakes and drinking responses to hypertonic and dehydrational stimuli, suggesting that they have accentuated thirst in comparison with older age groups. There were no differences in daily fluid intake from 6–24 m.o.; however, drinking responses to i.p. injection of hypertonic 0.4 mol/liter NaCl gradually declined over this period so that in 24-m.o. rats the response was only half that of 6-m.o. rats. Water intake after 24-h water deprivation also declined gradually over 24 months. Drinking responses to hypovolemia induced by s.c. injection of colloid (polyethylene glycol) were unchanged in 6- to 15-m.o. rats, then declined precipitously in 18- to 24-m.o. rats. Drinking responses to s.c. Ang II or s.c. isoproterenol were not reduced in 24-m.o. rats, nor was the drinking associated with feeding. Therefore, there are specific impairments of water intake in response to hypotonicity and hypovolemia in aged MW rats, but Ang-related drinking is not reduced. Like aged humans, aged MW rats exhibit high plasma atrial natriuretic peptide levels and impaired cardiovascular reflexes that could contribute to the impairment of thirst with age.

Adequate water intake is essential for life in most mammals. Thirst provides a specific motivational stimulus to ingest fluids when bodily fluid water content falls or its tonicity rises. Thus, factors that impair the thirst mechanism can have severe deleterious effects, leading to dehydration and even death. A significant proportion of aged human subjects has an impairment of thirst, particularly after a period of fluid deprivation (1, 2). Thus, they may be at risk for dehydration and in conditions of hot weather become severely dehydrated and at risk for heat stroke and circulatory collapse (3).

Whether thirst in general is reduced in aged humans, or there is a specific deficit in thirst in response to a particular dipsogenic stimulus such as hypertonicity, hypovolemia, or some other factor is unresolved. Fluid deprivation, resulting in dehydration that entails both hypertonicity and hypovolemia of extracellular fluid, usually has resulted in lower thirst ratings in elderly subjects (1, 2, 4–7). However, diminished thirst and reduced water drinking in response to i.v. infusion of hypertonic saline in elderly compared with younger subjects has been reported by some (8, 9) but not all investigators (10, 11). The effect of aging on human thirst in response to hypovolemia per se appears not to have been investigated.

Some strains of rats have been surveyed as suitable models for studying the effect of aging on thirst. Aged rats of the Brown Norway (BN) but not the Fischer 344 (F344) or Sprague Dawley (SD) strains drink less in response to a hypertonic or dehydration stimulus than young animals (12–15). The effect of hypovolemia induced by diuretic treatment has been studied in SD, F344, and BN strains, and intake of saline in response to such fluid and electrolyte loss was depressed in some aged animals (13). Whether hypovolemic thirst was depressed with age was difficult to ascertain in these studies because measurement of water intake independently of NaCl intake was not made. Similar to elderly humans, aged BN (13) but not aged SD or F344 (12) rats have impaired fluid and saline intakes in response to water deprivation.

Aged Munich Wistar (MW) rats, like some other rat strains, develop some features characteristic of aging humans (16–18) in that they have high circulating atrial natriuretic peptide (ANP) levels and impaired cardiovascular reflexes compared with younger MW rats (R.L.W. and C.J.T., unpublished data). As well, most strains of rats examined show a depressed activity of the renin angiotensin (Ang) system as they age (19, 20). Each of these factors may influence thirst and water intake in rats (21–23) and humans (7, 24–26). Consequently, we have studied the effect of aging on water drinking in response to several different dipsogenic stimuli in the MW strain of rats because they appear to have similar changes with age in several of the related cardiovascular and endocrine responses that occur in elderly humans. In particular, we aimed to test the effect of increasing age on both osmotically stimulated and hypovolemic thirst. In addition, we have measured drinking responses to pharmacological dipsogenic stimuli such as peripherally administered isoproterenol or Ang II, as well as the physiological stimuli of water deprivation, feeding, and normal daily intake, to shed light on how aging may impair thirst.

Results

Daily Food and Water Intakes and Body Weights of MW Rats 3–24 Months Old (m.o.). Body weight increased rapidly during the first 3–4 months of life, then stabilized so that after 6 months of age body weight had virtually reached a plateau, increasing very little during the subsequent 21 months (Fig. 1). Although the absolute levels of food and water intake each day varied little across the ages 3–24 months, on a body-weight basis, young 3-m.o. MW rats had the greatest food and water intake of any age group (Fig. 1). After this time, there was little change in daily food or water intake during the subsequent 21 months (Fig. 1).

Drinking Responses to Hypertonic Saline Injection. Intrapерitoneal injection of 0.4 mol/liter NaCl (2 ml/100 g), which increases the tonicity of body fluids, usually caused water drinking within 30 min in all age groups, which continued during the subsequent 90

Conflict of interest statement: No conflicts declared.

Abbreviations: MW, Munich Wistar; m.o., months old; BN, Brown Norway; F344, Fischer 344; SD, Sprague Dawley; Ang, angiotensin; PEG, polyethylene glycol; ANP, atrial natriuretic peptide.

†To whom correspondence may be addressed. E-mail: mmck@hfi.unimelb.edu.au or ddenton@unimelb.edu.au.

© 2006 by The National Academy of Sciences of the USA
The largest water intake, both in absolute terms and on a body-weight basis, occurred in the youngest (3 m.o.) animals (Fig. 2). These young adult rats drank $\sim$30% more water than those tested when 6 m.o. There was a trend for such osmotically stimulated drinking to diminish further during the subsequent 18 months (Fig. 2). Thus, 24-m.o. MW rats drank $\sim$50% less water in response to this stimulus than did 6-m.o. rats ($P < 0.01$). There was comparatively little water drunk by 6-m.o. rats ($n = 5$) or 24-m.o. rats ($n = 5$) after control i.p. injection of isotonic saline (1.7 ± 1.0 and 0.5 ± 0.7 ml/kg per 2 h, respectively). Blood samples from the tail vein were obtained before and 1 h after injection of the 0.4 mol/liter NaCl solution in 3- and 24-m.o. rats not tested for drinking responses, and plasma Na and Cl concentrations were measured. Plasma Na increased from 141.0 ± 1.2 to 146.8 ± 1.0 mmol/liter in 3-m.o. MW rats ($n = 4$) and from 143.3 ± 1.0 to 148.5 ± 2.1 mmol/liter in 24-m.o. rats ($n = 4$). Plasma Cl concentration increased from 102.8 ± 2.2 to 110.5 ± 1.9 mmol/liter in 3-m.o. rats, whereas it increased from 101.8 ± 1.0 to 109.3 ± 2.2 mmol/liter in 24-m.o. rats. The increases of plasma Na and Cl concentrations were not significantly different between the two age groups.

Drinking Responses to Water Deprivation for 24 Hours. The amount of water drunk on a body-weight basis in a 2-h period subsequent to 24 h of water deprivation was largest in the 3-m.o. MW rats (Fig. 3). Indeed, water consumption was approximately one-third more in these animals compared with 6-m.o. MW rats. After 6 months of age, there was a trend for deprivation-induced drinking to fall further so that it was significantly less in 10- and
At both ages, the increases in packed cell volume in 3-m.o. MW rats were significantly greater than in 15-m.o. rats than in 6-m.o. rats (Fig. 3). By 18 and 24 months, the drinking responses were approximately half those of 6-m.o. MW rats (Fig. 3). The loss of body weight expressed as a fraction of the initial body weight before the 24-h period of water deprivation was similar in all age groups (Fig. 3). The food intake of the 3-m.o. group was significantly greater than that of all other groups during the period of water deprivation, but food intake of the other groups was similar (Fig. 3).

**Ang-Stimulated Drinking.** Drinking in response to s.c. injection of Ang II was tested at several ages in rats 3–24 m.o. Rats of all ages drank water within a few minutes of injection of Ang II, and the total intakes after 2 h were not significantly different at any age tested (Fig. 4). There was little or no water intake after control s.c. injections of vehicle solution in 6-m.o. rats (n = 5) and 24-m.o. rats (n = 5) (0.2 ± 0.2 and 0.7 ± 0.9 ml/kg per 2 h, respectively).

**Isoproterenol-Stimulated Drinking.** The β-adrenergic agonist drug isoproterenol stimulates drinking in rats mainly by elevating circulating endogenous Ang II levels (27, 28). Drinking in response to s.c. injection of isoproterenol (25 mg/kg s.c.) was tested in MW rats at four different ages. We did not observe any significant difference in total water intake during the 2 h after treatment in any of the four age groups tested. The intakes of water by 3-, 6-, 15-, and 24-m.o. groups in the 2 h after isoproterenol injection were 17.3 ± 5.7 (n = 8), 15.1 ± 3.9 (n = 6), 18.8 ± 7.0 (n = 5), and 13.2 ± 4.5 (n = 7) ml/kg, respectively.

**Drinking in Response to Hypovolemia.** Subcutaneous injection of the high molecular weight colloid polyethylene glycol (PEG) results in a hypovolemic state in the rat because of the sequestration of isotonic extracellular fluid under the skin and stimulates water intake over several hours (29). Water drinking during the 6 h subsequent to s.c. injection of PEG (30%, 1.5 ml/100 g body weight; Sigma) was tested in MW rats, 3–24 m.o. (Fig. 5). The animals usually began to drink water 2–4 h after the injection of PEG and ingested ∼15 ml/kg of fluid during the 6 h of observation, except for the 18- and 24-m.o. rats, which drank significantly less (approximately half as much) than rats aged 3–15 months (Fig. 5). There was little, if any, intake of NaCl solution during this time in 6-m.o. rats administered s.h. s.c. injection of isotonic saline. In two groups of MW rats, 3 and 18 m.o., not being tested for their drinking responses, we measured the change in packed cell volume and plasma protein concentration in tail vein blood 3.5 h after the injection of PEG. At both ages, the increases in packed cell volume in 3-m.o. MW rats (n = 4) and 18-m.o. MW rats (n = 5) were similar (47.3 ± 1.1 to 50.1 ± 2.1 and 46.3 ± 1.5 to 50.7 ± 1.6% red blood cells, respectively) as were the increases in plasma protein concentration (8.4 ± 0.2 to 9.0 ± 0.4 g/dl in 3-m.o. rats and 8.6 ± 0.3 to 9.1 ± 0.3 g/dl in 18-m.o. rats). There was little NaCl intake during the 6 h of observations in any age group.

**Drinking Associated with Feeding.** In rats deprived of food, but not water, for 24 h the two oldest groups tested (15 and 24 m.o.) ate significantly less food relative to body weight compared with 3-, 6-, or 10-m.o. rats during the 3 h after the presentation of food to them. However, the volume of water drunk per g of food eaten was not significantly different between any of the age groups, being 0.61 ± 0.35 (n = 7) and 0.46 ± 0.28 (n = 5) ml/g of food in 3- and 6-m.o. rats and 0.54 ± 0.3 (n = 6), 1.01 ± 0.53 (n = 7), and 0.65 ± 0.23 (n = 9) ml/g of food in 10-, 15-, and 24-m.o. MW rats, respectively.

**Discussion**

Several observations were made in MW rats that warrant comment. First, young adult MW rats (the 3-m.o. group) had an avid thirst mechanism compared with older MW rats, including 6-m.o. rats that would not be considered to be in the aged category. Second, there is an effect of advanced age (18- to 24-m.o. rats) on water drinking in response to some, but not all, dipsogenic stimuli in this strain of rat. Third, although there appears to be a gradual reduction in osmotically stimulated drinking over 24 months in MW rats, there is an abrupt diminution of drinking in response to hypovolemic stimuli at advanced ages (18–24 months). Fourth, drinking responses to Ang-related stimuli or drinking associated with feeding are not inhibited with advanced age.

Clearly, young adult rats (3 m.o.) show the most striking dipsogenic responses and the greatest daily intakes of water (and food) of all of the age groups. These rats are also likely to be the most active and rapidly growing animals, and as such they may not be surprising that their fluid intakes are commensurate with a relatively larger food intake and probably larger metabolic rate. A well primed thirst mechanism may also be advantageous for younger rats because a greater surface-area-to-body-weight ratio may render them more prone to evaporative dehydration. In view of the avid drinking responses of the 3-m.o. group, we have opted in the main for using 6-m.o. animals as the baseline for comparison of dipsogenic responses in older rats. At 6 months of age, MW rats are close to their maximum body weight, and their weight is not significantly different from 24-m.o. MW rats. As well, daily food and water intakes have stabilized, and most
Dipsogenic responses are virtually unchanged from this age until they are 15 m.o. In previous studies of SD, F344, and BN rats, ages of the young adult rat groups were 5–7, 5–7, and 4–7 months of age, respectively (12–15). Thus, utilization of the 6-m.o. group of rats as the comparative nonaged group is consistent with these previous studies and readily facilitates comparisons between the different strains of rat.

In contrast to aged SD and F344 rats (12), but similar to BN rats (13), aged MW rats show decreased water drinking in response to acute systemic hypertonicity. Old rats may excrete hypertonic loads more rapidly than younger animals (12), suggesting the possibility that the thirst osmoreceptor may have been exposed to a lesser osmotic stimulus in the aged rats, this being the reason for the large reduction of drinking in response to hypertonic saline. However, reduced drinking in response to peripheral administration of hypertonic saline does not appear to be caused by reduced osmotic stimulation caused by altered excetration, absorption, or distribution of the injected hypertonic NaCl load, because similar increases in plasma Na concentration were measured in young and old MW rats at 1 h after the injection of hypertonic NaCl. Our data also suggest that there is a gradual rather than abrupt reduction of osmotically stimulated thirst as MW rats age from 6 to 24 m.o., which is also borne out by the gradual reduction in dehydration-induced drinking (Fig. 3). The underlying cause of this gradual diminution of osmotic thirst with age remains to be determined.

In contrast to this gradual reduction of osmotically stimulated drinking, there was a more precipitous reduction of water intake in aged MW rats in response to hypovolemia, induced by sequestration of isotonic extracellular fluid sc. after s.c injection of PEG (29). Similar dipsogenic responses to s.c. PEG were observed in MW rats from 6 to 15 m.o. From age 18 months, an abrupt and pronounced reduction in response was observed, but judging from the similar increases of packed cell volume and plasma protein concentration in 3- and 18-m.o. MW rats, the hypovolemic stimuli were similar. The gradual reduction of osmotically stimulated drinking compared with the more abrupt decline in hypovolemic drinking suggests that different mechanisms may be responsible for the age-related inhibitory influences on these two dipsogenic stimuli.

Considerable evidence from studies in the rat shows that hypovolemic thirst is mediated in part by activation of the peripheral renin–Ang system (30). Thus, elevated plasma Ang II levels that result from hypovolemia act on specific Ang II AT1 receptors in the subfornical organ to increase thirst and fluid intake (23).

However, other factors besides Ang also play a role in PEG-induced drinking, because it is not abolished in nephrectomized rats (31, 32), and although reduced, it also is not abolished by treatment with Ang-converting enzyme inhibitors or an Ang receptor antagonist (33). There is evidence that the renin–Ang system becomes depressed in aged rats and humans (19, 20, 34), suggesting that this factor could be a cause of the depressed water intake in aged MW rats, in response to both PEG-induced hypovolemia and dehydration. However, isoproterenol-induced drinking, which depends on endogenous Ang II formation (27), was not significantly reduced in old rats, suggesting that in the MW rat strain, reduced activity of the renin–Ang system is not a major factor in depressed drinking response.

Not surprisingly, drinking after 24 h of water deprivation, which is associated with both systemic hypertonicity and hypovolemia, and therefore dehydration of both intracellular and extracellular compartments (7), was also reduced in aged MW rats. Although Rowland et al. (12) did not observe aged SD or F344 rats to have a reduced thirst to dehydration, BN and F344/BN did show reduced water intake in response to dehydration (13–15). Thus the MW rats of the present study and BN and F344/BN rats exhibit analogous responses to aged humans who have consistently shown an impaired thirst rating and fluid intake after overnight water deprivation (1, 2, 7). There was a steady reduction of the response from age 3 to 18 months of age in MW rats. The changes in body weight and the food intakes of the 24 h of water deprivation were not different between the 6-m.o. rats and the 10-, 15-, 18-, and 24-m.o. groups of animals, making it likely that all groups of MW rats experienced similar degrees of dehydration, yet the four eldest groups showed diminished water drinking responses.

Not all dipsogenic responses were reduced in aged MW rats. Robust drinking response to s.c. injection of Ang II was observed in MW rats of all ages. This result is consistent with observations of Rowland et al. (12) who studied F344 and SD rats. Although Silver et al. (14) reported that aged (24 m.o.) F1 progeny F344/BN rats drank less in response to Ang II than did young (3 m.o.) rats (14), this result may reflect the avid thirst of young animals. Those authors also showed that 24-m.o. F344/BN rats had similar response to Ang II as 12-m.o. animals, which suggests that advanced age does not impair Ang II-stimulated drinking in the strains of rat studied to date. The data from all four strains of rats that have been studied suggest that the central mechanisms of thirst downstream from stimulation of the subfornical neurons by Ang II are intact in aged rats. There is one proviso, however, regarding this assertion, in that the arterial baroreceptor reflex has been found to be significantly less effective in aged MW rats (R.L.W. and C.J.T., unpublished observations). Under normal circumstances, baroreceptor activation has inhibitory effects on Ang II-stimulated drinking (21), and reduction of arterial pressure potentiates the Ang-II-stimulated dipsogenic response (35). Therefore, if there were reduced signals from arterial baroreceptors reaching central neural pathways driving thirst in aged rats injected with Ang II, a greater drinking response to Ang II might be expected.

The daily 24-h water intake relative to body weight of aged rats also was not different to that of 6- to 15-m.o. rats, suggesting that under normal nonchallenged conditions aged rats maintain fluid balance adequately, which is supported by similar plasma Na concentrations in aged and MW young rats. In general, aged humans also maintain daily fluid intake, usually as a result of drinking fluids with meals and by habit (7, 36).

What could be the mechanism of impaired drinking responses to hypertonicity or hypovolemia in aged rats and humans? As mentioned already, there may be more than one factor involved. If the release of renin and generation of circulating Ang II is reduced in aged animals, as Thunhorst and Johnson (13) have pointed out, this could reduce drinking responses in conditions (e.g., PEG-induced hypovolemia, dehydration) where the renin–Ang system is activated (37). However those authors did not dismiss the possible influence of other factors, and it would seem unlikely that depressed Ang II levels are the cause of reduced osmotically stimulated thirst because hypertonic saline treatment will inhibit renin release and Ang II formation. Water drinking in response to isoproterenol treatment in rats depends on renal renin secretion and endogenous Ang II formation (27). Thus, our observation that isoproterenol-induced drinking was not depressed in the oldest MW rats argues against a role for reduced renin–Ang activity being the cause of age-induced reduction of drinking in the MW strain of rats.

A second consideration in this regard are the high circulating levels of ANP that occur in plasma of aged rats and humans (16, 38). We have also measured plasma ANP in 24-m.o. MW rats and found them to be approximately double those of 6-m.o. MW rats (R.L.W. and C.J.T., unpublished data). Interestingly, Reckelhoff et al. (39) did not observe elevated basal or hypertonic saline-stimulated plasma ANP levels in aged SD rats, and aged rats of this strain do not show impaired drinking responses to hypertonic saline treatment (12). As ANP can have inhibitory effects in the rat on...
dehydration and Ang II-induced drinking (20), it is possible that the high levels of ANP in aged MW rats are the cause of the reduced drinking in response to systemic hypertonicity.

A third factor that should also be considered regarding impaired thirst in aged rats and humans, particularly in regard to hypovolemic thirst, is the impairment of cardiovascular reflexes that occurs with aging. Both the arterial baroreceptor reflex and cardiac vagal afferent-driven “high pressure” baroreceptor and chemosensitive (Bezold–Jarisch) reflexes are impaired in reduced vs. young MW rats (C.J.T. and R.L.W., unpublished data), and reflexes arising from the arterial baroreceptors and cardiopulmonary afferents are reduced in aged humans (18). Afferent neural signals from stretch receptors in the heart, and hypotension per se, independent of any activation of the renin–Ang system, may play important roles in the stimulation of thirst in the rat (23, 30, 40). If PEG-induced hypovolemia results in reduced arterial pressure, the facilitatory influence of hypotension on thirst in younger animals may be impaired in aged MW rats. Thus, as cardiac reflexes are impaired generally in the aged (17, 18), it is possible that dysfunctional afferent signals to the CNS from both high- and low-pressure sensors in the heart may have a deleterious influence on thirst responses. As changes in cardiac reflexes do not come into play until fairly advanced age, the abrupt decline in hypovolemic thirst with advanced age would be consistent with such a proposal. Clearly, this area of investigation, along with the role of elevated ANP levels in the aged, needs further investigation.

In conclusion, aged MW rats show a reduction in water drinking in response to both hypertonicity and hypovolemia, but not to injected Ang II, compared with young adult or middle-aged rats. On the other hand, young MW rats appear to have an enhanced thirst mechanism that may be advantageous in overcoming body fluid losses. The impaired drinking in response to hypertonic saline administration and water deprivation, the relatively adequate daily water intake under nonchallenged conditions, the high circulating levels of ANP, and impaired cardiovascular reflexes suggest that MW rats may be excellent models for studying mechanisms of thirst impairment seen in humans with aging. In particular, the roles of depressed cardiac reflexes, depressed renin–Ang system, and elevated ANP levels in depressed thirst of aged rats are ripe for further investigation. In addition, the apparent enhanced thirst in the young also requires a mechanistic explanation that may not be the converse of the causes of thirst impairment with advanced age.

Methods

All experimental protocols and procedures were approved by the Animal Ethics Committee of the Howard Florey Institute, which adheres to the Code of Practice of the Australian National Health and Medical Research Council for the care and use of experimental animals. Rats of the MW strain were used. They were obtained from the Animal Resources Centre in Perth, Western Australia when they were 2–2.5 months of age, then housed individually in wire high-topped plastic boxes that adhered to the Code of Practice of the Australian National Animal Ethics Committee of the Howard Florey Institute, which requires a mechanistic explanation that may not be the converse of the causes of thirst impairment with advanced age.

Experimental Protocols. Each rat was submitted to several different dipsogenic stimuli during its life during daylight hours; however, no rat was submitted to a particular dipsogenic stimulus on more than one occasion during its lifetime. Thus, for a specific dipsogenic stimulus, each rat was naive when tested, so that there was no longitudinal study of a particular dipsogenic stimulus in any individual animal. The different ages chosen for investigation were usually 3, 6, 10, and 15 or 16, 18, and 24 months of age. The sequence of administration of the various dipsogenic stimuli was random over the course of a rat’s life, so that different rats were given different stimuli at particular ages. In some rats, dipsogenic tests were not commenced until they were 15 or 18 m.o. As there were some deaths (caused by tumors, natural attrition) over the course of the experiments, not all rats received all dipsogenic stimuli, and some rats were administered more than one dipsogenic test at a particular age (with a tolerance of ±3 weeks). Results obtained from a rat within 3 months of the detection of a tumor or growth in that rat were discarded. Body weight of rats was regularly measured, and daily food and water intakes were occasionally obtained in some rats by averaging intakes over 2–3 successive days.

Dipsogenic Stimuli. The volumes of water drunk in response to several different dipsogenic stimuli were measured in MW rats at ages that ranged from 3 to 24 months. Volumes of water drunk from graduated drinking burettes were measured to the nearest 0.1 ml in these tests. The dipsogenic stimuli that were administered to MW were (i) an acute increase in plasma toxicity by i.p. injection of hypertonic saline, (ii) dehydration resulting from water deprivation for 24 h, (iii) s.c. injection of the dipsogenic hormone Ang II, (iv) administration of the β-adrenergic agonist isoproterenol s.c. that stimulates drinking mainly by elevating endogenous blood Ang II levels (27), (v) hypovolemia produced by s.c. injection of the high molecular weight colloid PEG that results in sequestration of isotonic extracellular fluid under the skin (28, 29), and (vi) water drinking associated with feeding after a period of food deprivation.

Hypertonic Saline. Rats were slowly (over ~30 s) injected i.p. with 2 ml/100 g of body weight of 0.4 mol/liter NaCl warmed to 37°C. They were returned immediately to their cages, and the volume of water drunk was measured at 30-min intervals for 2 h. Drinking responses during the same 2-h period to control i.p. injections of isotonic 0.15 mol/liter NaCl (2 ml/100 g of body weight) were made in two of the groups, 6- and 24-m.o. MW rats. In a separate experiment in rats of the 3- and 24-m.o. groups in which water was withheld so that no drinking occurred, 1-ml samples of blood were obtained from the tail tip into a tube containing 5 μl of heparin before and 1 h after the i.p. injection of 0.4 mol/liter NaCl. Plasma was obtained by centrifugation of the sample, and plasma concentrations of Na and Cl were measured by ion selective electrode (ABL System 625; Radiometer, Copenhagen).

Water Deprivation. On the first morning of the experiment, the body weight of rats was measured, a measured portion of food was placed in the cage, and water was removed from the cage for the subsequent 24 h. The next morning, the body weight of rats was measured again, the remaining food was removed from the cage and weighed, and a burette of fresh tap water was placed in the cage. Water intake during the subsequent 2 h was measured at 30-min intervals.

Peripheral Ang II. Rats were given a s.c. injection of Ang II (Auspep, Melbourne) at 100 μg/kg. Water intake was measured at 15-min intervals for 1 h, then again at 2 h. The effect on water intake of a control injection of vehicle (isotonic 0.15 mol/liter NaCl, 0.05 ml/kg s.c.) was tested in 6- and 24-m.o. rats.

Peripheral Isoproterenol. Rats were given a s.c. injection of isoproterenol (Abbott) at 25 mg/kg. Water intake was then measured at 15-min intervals for 2 h.
Subcutaneous PEG. Rats that were supplied with both water and 0.5 mol/liter NaCl in the cage were administered an injection of 1.5 ml 10% (wt/vol) PEG (molecular weight 10,000–20,000; Sigma) under the skin of the back after a s.c. injection of local anesthetic (xylocaine) into that region. Intakes of water and NaCl were then measured during the injection of local anesthetic (xylocaine) under the skin of the back after a s.c. injection of 1.5 ml PEG (molecular weight 10,000–20,000; Sigma) under the skin of the back after a s.c. injection of local anesthetic (xylocaine) into that region. Intakes of water and NaCl were then measured during the subsequent 6 h at 2-h intervals. In a separate experiment in rats aged 3 and 24 m.o. where water and NaCl solution were withheld, a blood sample of 1 ml was obtained from the tip of the tail before making an identical s.c. injection of PEG as described above, then another 1-ml blood sample was obtained from the tail tip at 3.5 h after the injection of PEG. Packed cell volume was obtained by centrifugation of blood in microcentrifuge tubes, plasma was obtained by centrifugation of the remainder of the samples, and the plasma protein concentrations were determined by measuring the refractive index of the plasma.

Drinking Associated with Feeding. Commencing at midday, rats were deprived of food but not water for 24 h. At midday the next day, a weighed amount of food was placed in the cage, and the weight of food eaten and volume of water drunk in the subsequent 2 h were measured.

Statistical Treatment of Results. All results are expressed as mean ± standard deviation. Comparison of responses of different age groups to a particular stimulus was made by one-way ANOVA, followed by a multiple comparison test (Student–Neuman–Keuls). Changes in packed cell volume and plasma electrolytes and protein concentration were compared by Student’s t test.

This work was supported by National Health and Medical Research Council of Australia Project Grant 223206, the Robert J. Jr. and Helen C. Kleberg Foundation, the Harold G. and Leifa Y. Mathers Charitable Foundation, and the Search Foundation.