Water deprivation and the double-depletion hypothesis: common neural mechanisms underlie thirst and salt appetite

L.A. De Luca Jr.[1],
R.C. Vendramini[2]*,
D.T.B. Pereira[1]*,
D.A.S. Colombari[1],
R.B. David[1]*, P.M. de Paula[1]
and J.V. Menani[1]

[1]Departamento de Fisiologia e Patologia, Faculdade de Odontologia,
[2]Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas,
Universidade Estadual Paulista, Araraquara, SP, Brasil

Abstract

Correspondence

L.A. De Luca Jr.
Departamento de Fisiologia e
Patologia
Faculdade de Odontologia, UNESP
Rua Humaitá, 1680
14801-903 Araraquara, SP
Brasil
Fax: +55-16-3301-6488
E-mail: lucajr@foar.unesp.br

Water deprivation-induced thirst is explained by the double-depletion hypothesis, which predicts that dehydration of the two major body fluid compartments, the extracellular and intracellular compartments, activates signals that combine centrally to induce water intake. However, sodium appetite is also elicited by water deprivation. In this brief review, we stress the importance of the water-depletion and partial extracellular fluid-repletion protocol which permits the distinction between sodium appetite and thirst. Consistent enhancement or a *de novo* production of sodium intake induced by deactivation of inhibitory nuclei (e.g., lateral parabrachial nucleus) or hormones (oxytocin, atrial natriuretic peptide), in water-deprived, extracellular-dehydrated or, contrary to tradition, intracellular-dehydrated rats, suggests that sodium appetite and thirst share more mechanisms than previously thought. Water deprivation has physiological and health effects in humans that might be related to the salt craving shown by our species.

Introduction

Sodium appetite and thirst are recognized complementary motivational states that drive a dehydrated animal to refill its major fluid compartments. Here we review the fact that water deprivation, the most common and natural form of inducing dehydration, activates not only thirst, but also sodium appetite.

Thirst and sodium appetite results from water deprivation

The double-depletion hypothesis of thirst predicts that water intake occurs as a response to a water deficit generated by liquid loss from the two major body compartments, i.e., the intra- and extracellular compartments (1). Thus, combined contraction and increased tonicity of the extracellular fluid (ECF) generates signals through the renin-angiotensin system and osmoreceptors, respectively, that in turn combine centrally to produce thirst (1,2). The double-depletion hypothesis is seminal to link these two mechanisms once considered separate to explain thirst in water-deprived animals. An early crucial set of experiments testing such a link was the attempt to suppress thirst by providing an intragastric or intravenous

preload of the appropriate fluid in volumes predicted to be sufficient to refill each compartment, i.e., water for the intracellular compartment, and 0.15 M NaCl (i.e., isotonic solution) for the extracellular one (3,4). Under carefully controlled conditions, the experiments essentially showed that thirst induced by water deprivation, as measured by the amount of water intake, is not completely suppressed by either preload alone, but by the two combined.

However, water-deprived rats ingested large amounts of isotonic NaCl after they received water preloads equivalent to the total volume of water they normally ingest. The authors concluded that intracellular overhydration, as induced by the ingestion of only water, did not reduce thirst, but only the desire to ingest water (4). The authors apparently assumed that isotonic NaCl intake is related to thirst, as also proposed by others to explain why cell-dehydrated rats also ingest isotonic NaCl (5,6). The main implication of this conclusion is that thirst drives a rat to ingest not only water, but also salt. One may agree in part with this view, because water is not always the most preferred liquid compared to mineral solutions under conditions of selective acute dehydration of either the extracellular or the intracellular compartment occurring within hours (7). Moreover, the total amount of fluid ingested by water-deprived rats is strongly influenced by taste (8,9). However, there are two caveats about the view that thirst induces a desire to ingest water and salt, even if the salt is at isotonic concentration. First, according to what appears to be common sense, other investigators have related the expression of thirst only to water intake (10,11). Second, the early experiments might have overlooked the possibility that sodium appetite, or the selective ingestion of sodium solutions and increased hypertonic NaCl intake, also results from water deprivation.

Several hours of water deprivation lead to appropriate physiological alterations that initiate a typical sodium appetite. For example, water-deprived rats, rabbits, sheep, or humans have not only contraction of the ECF, but also a negative sodium balance that results from a mechanism to buffer the increase in extracellular tonicity (12,13). The first suggestion that water deprivation induces sodium appetite was based on these findings. When offered different types of mineral solutions simultaneously, water-deprived rats showed preference for sodium compared to other mineral solutions (14). This led us to ask whether angiotensin II mediates water deprivation-induced sodium appetite (15), as it does in the classic sodium depletion models such as adrenalectomy or diuretic injection combined with sodium-free food (16,17). However, water-deprived rats may behave non-specifically by ingesting any type of fluid that becomes immediately available to them, as suggested by pilot experiments in our laboratory and the literature (8), making it hard to know when thirst ends and sodium appetite begins.

In order to properly distinguish thirst from sodium appetite, we developed a protocol, the water-depletion and partial ECF-repletion protocol or WD-PR, in which thirst is quenched by allowing the rat to drink from a burette filled with water prior to any access to sodium (15). Then, as water intake fades to nil, a solution of NaCl is provided in another burette and a sodium appetite test begins. The intake of 0.3 M NaCl in the sodium appetite test increases as a function of water deprivation period as shown at Figure 1. Animals that are not water deprived ingest less than 0.5 mL of this salt solution during the test. Preliminary unpublished data from our laboratory (Leite AS, Pereira DTB, Menani JV, De Luca LA Jr) are also consistent with the earlier proposal that water deprivation induces a preference for sodium (14) because mineral ingestion during this sodium appetite test is much in the form of sodium solutions.

Crucial physiological and neurological

alterations in WD-PR associated with the subsequent ingestion of a sodium solution, in the sodium appetite test, are listed in Table 1 (18). Notice the negative sodium balance that results from reduced standard-food intake and from intracellular dehydration-induced natriuresis. The increase in plasma renin activity induced by hypovolemia remains elevated after thirst satiation; this elevation correlates with c-Fos expression in the subfornical organ and, partially, in the lamina terminalis. Neurons in these two structures are activated by angiotensin II and belong to encephalic circuits that subserve sodium appetite mediated by angiotensin II (19-24). The WD-PR protocol is instrumental for the execution of acute tests aimed at understanding the mechanisms of sodium appetite because it allows one to distinguish thirst from sodium appetite originating from the same treatment, i.e., water deprivation. Under these conditions, we have shown that blockade of converting enzyme or central angiotensin II receptors strongly inhibits the ingestion of hypertonic sodium solution during the sodium appetite test (15).

Therefore, water deprivation relates not only to thirst, but also to sodium appetite, thus leading to a new equation where water deprivation = thirst + sodium appetite.

Thirst and sodium appetite: two sides of the same coin?

So much for water deprivation, but, considering water deprivation = double depletion, can one also conclude that double depletion = thirst + sodium appetite? The classical findings in this field of study say no, because, whereas extracellular dehydration facilitates, intracellular dehydration inhibits sodium appetite. However, the combination of earlier data with more recent experiments may impose a challenging "perhaps".

As mentioned above, since the early experiments testing the double-depletion hypothesis it has been known that animals in the early stages of dehydration, whether extra- or intracellular, ingest both water and isotonic NaCl. However, ingestion of isotonic NaCl is not traditionally considered to represent sodium appetite. First, because animals in early extracellular or intracellular dehydration also ingest water, but reject hypertonic NaCl (4,7,25,26). Second, neither water nor sodium solution is preferred by early extracellularly or intracellularly dehydrated rats; whereas the former may ingest equal amounts of sodium and non-sodium solutions, as well as water, the latter show an uncanny preference for a potassium solution (7). Third, ECF hypertonicity is classically
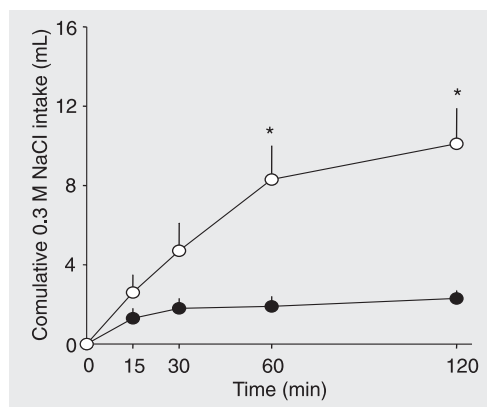

Figure 1. Cumulative 0.3 M NaCl intake during a sodium appetite test by rats that had previous access only to water (partial extracellular fluid repletion, PR) after 24- (WD 24-PR) or 36-h (WD 36-PR) water deprivation (WD). WD 24-PR (filled circles, N = 10) and WD 36-PR (open circles, N = 12). Hydrated animals ingest less than 0.5 mL 0.3 M NaCl during a period equivalent to the sodium appetite test. $P < 0.05$ compared to WD 24-PR ($F_{(1,80)}$ two-way repeated measures ANOVA and *post hoc* Student-Newman Keuls test).

Table 1. Fluid-electrolyte balance, hormonal activity, neuronal activity, blood pressure, and behavior of water-deprived rats prior to and after ingestion of water in the water-depletion and partial extracellular fluid-repletion (WD-PR) protocol (from Ref. 18, with permission).

Prior to water intake (WD)	After only water intake (PR)
Water deprivation	Partial volume repletion
Negative sodium balance	Negative sodium balance
Hypertonicity	Hypertonicity and thirst removed
Anorexia	Partial hypovolemia
Hypovolemia	RAS activity still highly elevated
Enhanced RAS activity	Normal mean arterial pressure
Normal mean arterial pressure	Removal of Fos-ir in SON
Fos-ir in SON	Partial reduced Fos-ir in lamina terminalis,
Fos-ir in lamina terminalis	but not in SFO

Partial extracellular fluid volume repletion is achieved by water intake and corresponds to the animal's internal status just prior to access to a sodium solution in the sodium appetite test. RAS = renin-angiotensin system; Fos-ir = Fos-like immunoreactivity; SON = supraoptic nucleus; SFO = subfornical organ.

linked to inhibition of hypertonic NaCl intake (27).

Nevertheless, ECF hypertonicity might also induce hypertonic NaCl intake under special conditions. This was first suggested by studies showing enhanced hypertonic NaCl intake in animals either made hyponatremic with systemic hypertonic mannitol or injected systemically with hypertonic NaCl when hypovolemic (28,29). Central inactivation or blockade of either central oxytocin or atrial natriuretic peptide receptors of these animals not only reduced the inhibition of hypertonic NaCl intake, but also induced hypertonic NaCl intake at amounts far above control. This enhanced hypertonic NaCl intake is similar to that shown by hypovolemic animals that have also their central oxytocin receptors blocked (29, but also see 30). More recently, it has been shown that enhanced hypertonic NaCl intake also occurs when serotonin receptors are blocked in the lateral parabrachial nucleus of either water-deprived, selectively extracellularly dehydrated or selectively intracellularly dehydrated rats; no hypertonic NaCl intake occurs in hydrated animals bearing this type of blockade (31-33).

Taken together, these data regarding inhibitory hormones and an inhibitory nucleus suggest that inhibitory circuits are a determinant factor of hypertonic NaCl intake, particularly in hyperosmotic animals. If so, it is possible that ECF hypertonicity and angiotensin II activate circuits that subserve thirst, and, contrary to common sense about ECF hypertonicity, also induce sodium appetite in the water-deprived rat, but the inhibitory factors activated by hypertonicity also produce strong inhibition of sodium appetite. Thus, a water-deprived rat that has immediate access to a salty solution may ingest it because it is searching for a liquid. If the animal first ingests only water instead, the inhibitory hypertonicity is removed, leaving only the facilitatory mechanisms, like angiotensin II, to act on sodium appetite. In addition, the more a salty solution is diluted, the less an inhibitory system interferes, thus allowing, for example, a cell-dehydrated rat with an intact lateral parabrachial nucleus to ingest isotonic NaCl as the most concentrated sodium solution in addition to water (4,34).

The possibility that hypertonicity and angiotensin II induce thirst and sodium appetite in the rat resembles the behavior of marine fish that respond to both types of dehydration by ingesting salty water (35) and thus one may ask whether some of the neural basis of hydromineral intake in mammals was not inherited from their marine ancestors, and whether thirst and sodium appetite were the same thing in the past.

Water deprivation, emergency reactions and addiction

Water deprivation induces global responses involving not only the drive to ingest water and salt, but also activation of systems that act to conserve volume and guarantee normal tissue perfusion. Enhanced circulating angiotensin II, vasopressin and sympathetic tone contribute to avoid a decrease in arterial pressure under reduced extracellular volume (36,37). Again, hormones may interact positively with hypertonicity. The increase in plasma osmotic pressure during water deprivation is not only important for vasopressin secretion, but also necessary for the increased sympathetic output that helps to sustain arterial pressure (38,39).

Water deprivation also enhances the secretion of glucocorticoid, a hormone that helps the animals to cope with stress and that is likely to interact with angiotensin II to increase water and sodium intake (40-42). Water-deprived humans have similar physiological alterations and this species has learned that a guarantee against dehydration is to secure water resources and to adjust water and salt intake in order to avoid exces-

sive water-lack hypovolemia or water-over-consumption hyponatremia (43-45). Some societies may still take water, and even salt, for granted, but this is not the rule all over the world because of either regional scarcity or reduced economic power (44). Nevertheless, whatever society is considered, a phenomenon called voluntary dehydration may be chronic in humans, and one can only speculate as to what consequences this phenomenon plus recurrent water deprivation may have for health and disease (46).

One guess is that water deprivation may contribute to the human craving for salt. Salt preference increases in humans subjected acutely to a protocol similar to the WD-PR (47). Repeated episodes of sodium depletion in rats lead to an enhanced sodium consumption that may share neural mechanisms with addiction (48,49) and recently we have shown that rats with a history of repeated episodes of water deprivation have increased daily sodium consumption (50).

Conclusion

Water homeostasis has had a major survival role in the terrestrial environment. The same is true for sodium homeostasis. Water deprivation challenges both and thereby elicits appropriate mechanisms that assure the replacement of water and sodium. Therefore, when studying dehydration-related states we should not forget that sodium appetite is elicited by water deprivation. Perhaps the application of WD-PR protocols in humans and animals may provide novel insights for this type of study.

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