Limits of brain tolerance to daily increments in serum sodium in chronically hyponatraemic rats treated with hypertonic saline or urea: advantages of urea

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SUMMARY

1. At present there is no consensus about the optimal management of hyponatraemia to prevent demyelinating brain lesions. We have evaluated in a large series of rats (n = 136) the protective role of urea for the brain in the treatment of severe chronic hyponatraemia. Urea (group I, n = 51) was compared with hypertonic saline in boluses (group II, n = 46) and with hypertonic saline in divided doses (group III, n = 39). Treatment was administered intraperitoneally over 48 h. The severity of brain lesions was assessed by histological scoring.

2. For 95% of the injured animals treated with hypertonic saline, brain lesions appeared for an absolute increment in serum Na⁺ concentration (ΔSₙa⁺) of 20 mmol day⁻¹. Above this limit neurological injuries gradually worsened, and beyond a transition zone (ΔSₙa⁺ ≥ 20 ≤ 23 mmol day⁻¹) 89% (group III) to 100% (group II) of the animals were injured. This limit can be reached rapidly, as attested by the comparable severity of brain lesions observed in group II (mean ΔSₙa⁺ 1 h after a bolus injection, 19 mmol/l) and in group III (mean ΔSₙa⁺ 1 h after an injection, 2 mmol/l), both groups achieving similar daily ΔSₙa⁺.

3. A correction above the threshold of 20 mmol day⁻¹ is as toxic during the first 24 h as during the second day of the treatment. This can be deleterious even if a mildly hyponatraemic level (≥ 120 < 129 mmol/l) is achieved after 48 h. The maximum ΔSₙa⁺ over 48 h tolerated by the brain can exceed 30 mmol/l (31–42 mmol/l), with a correction of serum Na⁺ concentration up to a normonatraemic level, provided that the successive daily ΔSₙa⁺ was not greater than 20 mmol/l. The severity of the pre-existing hyponatraemia does not influence the outcome of the correction phase.

4. We have confirmed the protective effect of urea against the development of brain damage when used to correct hyponatraemia. The threshold of tolerance to daily ΔSₙa⁺ after urea is identical to the limit defined for hypertonic saline, but a further rise in serum Na⁺ concentration (range of daily ΔSₙa⁺ > 23 < 30 mmol/l) leads to a significantly lower incidence (47%, P < 0.01 compared with group II and P < 0.02 compared with group III) and severity (P < 0.03 compared with group II and P < 0.005 compared with group III) of neurological complications.

Key words: antidiuretic hormone, central pontine myelinolysis, hypertonic saline, hyponatraemia, urea.

Abbreviations: CPM, central pontine myelinolysis; HS, histological score; MΔSₙa⁺, maximum daily absolute serum Na⁺ increment observed during 1 of the 2 days of the correction period; mΔSₙa⁺, absolute daily serum Na⁺ increment observed either the day before or the day after the MΔSₙa⁺ is achieved; ODS, osmotic demyelination syndrome; Sₙa⁺, serum Na⁺ concentration; ΔSₙa⁺, absolute increment in serum Na⁺ concentration.

INTRODUCTION

During the past decade attention has been drawn to the correction phase of chronic hyponatraemia as an important aetiological factor in central pontine myelinolysis (CPM), also called osmotic demyelination syndrome (ODS), in humans. Since the first reports suggesting that an 'overly rapid' correction of hyponatraemia might have been responsible for the subsequent clinical worsening observed in some patients [1], numerous efforts have been made in animal [2–8] and human [9–16] studies to determine precisely how rapidly, and by how much, the natraemia could be increased before ODS developed. However, there is no definite consensus about the
maximum tolerated increase in serum Na⁺ concentration (SNa⁺) over a given time (rate of correction). Besides, this parameter is not necessarily constant throughout the correction phase [7, 17]. Some difficulties result from the absence of carefully designed prospective studies in the human. The only report published [14] presents several controversial points [18]. The currently accepted approach [13], based upon clinical experience of the treatment of chronic severe (<120 mmol/l) hyponatraemia, is a ‘rapid correction’ with hypertonic saline (3.5% NaCl) by not more than 2 mmol h⁻¹ l⁻¹ and 25 mmol 48 h⁻¹ l⁻¹, until a level of 130 mmol/l is achieved [11, 13, 14]. An increase in SNa⁺ to normonatraemic or hyper- natraemic levels should also be avoided [14]. However, ODS has been reported with lower rates of correction [9, 12, 15] and mortality and morbidity of severe hyponatraemia have been probably overestimated [15]. Hence, slower rates of correction (0.50 mmol h⁻¹ l⁻¹, <12 mmol day⁻¹ l⁻¹), at least in chronic hyponatraemia, have been advocated [12, 15, 19]. On the other hand, the concept of a ‘mildly hyponatraemic level’ (120–130 mmol/l), proposed by some [11, 14] as a safe limit of correction, is also questionable [12, 19]. Experiments in animals have also failed in the attempt to define the minimum increment in SNa⁺ capable of producing therapeutic complications. Indeed, the results of studies in various species, such as dogs [3], rabbits [6] and rats [2, 4, 5, 7, 8], are difficult to interpret owing to the diversity of protocols used, the frequent lack of details of individual SNa⁺ evolution during the first 48 h of the correction [2, 3, 5] and the small number of animals used for each protocol [2, 3, 6]. In a recent study, Ayus et al. [8] have observed that brain lesions in rats developed only if the absolute increment in SNa⁺ (ΔSNa⁺) was >25 mmol/l in the first 24 h after correction with hypertonic saline. However, a number of points remain questionable and unanswered in that study. Data about brain susceptibility to the treatment beyond the first 24 h are also important in clinical situations and are lacking in experimental studies. Finally, there is some experimental evidence that the final correction up to mild hyponatraemia could also be toxic [3, 6, 7]. We have suggested in a preliminary experiment with a small number of chronic hyponatraemic rats [20] that urea may protect against ODS when used to correct the SNa⁺ rapidly (48 h). The protective effect of urea was established by comparing rats treated with urea with others receiving hypertonic saline once daily (in boluses). However, if saline administration in boluses is largely used in the animal models of ODS [2, 4–6, 8, 21, 22], it does not reflect the reality of the clinical setting where saline is generally more slowly infused. Moreover, differences in the kinetics of the rise in SNa⁺, which are due to the methods of correction could play a role in the tolerance to the treatment. Comparison with a model of slower hypertonic saline administration will test this hypothesis. Our aim was (1) to precisely define the threshold of maximum daily ΔSNa⁺ supported by the brain during a 48 h correction period; (2) to compare the kinetics of the rise in SNa⁺ and the effect on brain of correction with hypertonic saline either in boluses or in divided doses; (3) to evaluate the validity of the concept of a final ‘mildly hyponatraemic’ level of SNa⁺ preventing brain damage; (4) to test the role of the severity of the hyponatraemia before correction in the development of ODS; (5) to determine in a large number of animals to what extent urea could protect against ODS.

**MATERIALS AND METHODS**

**Animals**

A total of 259 male Wistar rats weighing 350–500 g were studied. Profound chronic hyponatraemia (SNa⁺ <115 mmol/l) was induced by subcutaneous injection of 0.5 units of vasopressin tannate in oil (Parke-Davis)/100 g body weight (injection site briefly massaged) and intraperitoneal injections of 2.5% (w/v; 140 mmol/l) D-glucose in water equivalent to 5% initial body weight. These injections were given twice daily (09.00 hours and 17.00 hours) on days 1 and 3 and once daily (12.00 hours) on day 2. No food or water was provided during this phase of the experiment [2].

**Correction of hyponatraemia (days 4 and 5)**

Three different groups were studied depending on the solutes used for the treatment of the hyponatraemic state and their mode of administration. In each group, the correction of hyponatraemia was performed on days 4 and 5. Rats had free access to the laboratory diet and water from day 6 to the time of death (day 8).

**Group I.** Ninety-three animals received a 5 mol/l (30%, w/v) solution of urea (freshly prepared each time) in 5% (w/v) D-glucose over 5 min. The solution was given twice daily by intraperitoneal injection (09.00 hours and 17.00 hours) over 2 days [20] at a dose of 0.5 ml/100 g body weight. The urea doses were divided in order to avoid symptoms related to uraemia [20, 23].

**Group II.** Eighty-five rats were treated with hypertonic saline (1 mol/l NaCl) at a dose of 2 ml day⁻¹ 100 g⁻¹ body weight as a single daily intraperitoneal injection over 2 min (at 09.00 hours) during 2 days [2].

**Group III.** In this group of 81 rats, the same total dose of hypertonic saline (1 mol/l; 2 x 2 ml/100 g body weight) was administered in 12 equal intraperitoneal injections (0.33 ml) given every 4 h over 48 h. Blood samples (0.50 ml) were collected via tail transaction under light ether anaesthesia for determination of SNa⁺. Measurement of SNa⁺ was made on day 1, day 4 (at the time of maximum hyponatraemia), day 5 (after 24 h of hyponatraemia), day 6 (after 48 h of hyponatraemia) and at the time of death (day 8). Additional animals, not included in the initial group of 259 rats, were treated in the same manner as groups I (n = 8), II (n = 13) and III (n = 10) but were submitted to repeated determinations of SNa⁺ in order to compare the kinetics of the rise in SNa⁺. Blood samples were collected on day 4 just before the first intraperitoneal injection and then 1, 5 and 8 h afterwards. SNa⁺ was measured by ion-selective analysis (Microlyte; Kone, Espoo, Finland).
Histology

Body weight was measured for all the animals on day 1, 4 and at the time of death. Clinical assessment of the neurological status was regularly made and any other abnormality was noted. All the rats that survived the experiment were decapitated on day 8. Afterwards brains were sectioned at six levels and were processed for light microscopy as previously described [20]. The sections were independently examined by two neuropathologists (O.P.; A.S.T.), who were unaware of the treatment the animals had received. A histological score (HS) was established to more quantitatively analyse the morphological differences observed between the different groups, using criteria of severity of the lesions for each examined area graded from 0 to 3 [4, 24]. This score encompasses both the intensity and the extension of the histological changes (0 = no lesion; 1 = focal changes or <30% of the anatomical site injured; 2 = >30%<60%, 3 >60%). The final score is represented by the sum of the scores (ranging from 0 to 3) of each anatomical area affected in rats all killed after the same length of time (day 8). The areas analysed were the same in all the rats. As the pathological analysis will demonstrate, the brain lesions are always symmetrical and therefore the score will account for only a half of the brain structure. We decided to exclude from the analysis rats which presented an overcorrected SNa+ on day 5 or 6 arbitrarily defined as SNa+ < 2 mmol/l over the initial SNa+ value. Overcorrection is a widely accepted risk factor of CPM [2, 3, 5, 6, 8, 9, 14]. Animals which exhibited respiratory arrest during light ether anaesthesia during each experiment and which survived were also excluded [14, 16, 19], as were rats which died before day 8. Brain analysis was not performed on these animals.

Statistical analysis

All values are expressed as means ± SD, except those for weight and for the kinetics of SNa+, which are expressed as means ± SEM. Statistical analysis for comparison of the various parameters between the different groups was performed by one-way analysis of variance for repeated measures, by conventional t-test or Wilcoxon’s test for between-group comparisons as appropriate [25] and by the χ² test.

RESULTS

Selected animals (n = 136)

In the present study, consisting of a total of 259 rats, 136 rats were finally available for further analysis. Forty-three rats (16%) died during the induction phase of hyponatraemia. Seven did not reach sufficiently severe hyponatraemia (SNa+ > 115 mmol/l). Thirty-eight rats (14%) died during the correction phase of hyponatraemia before day 8 [10 in group I (11%), 13 in group II (15%) and 15 in group III (19%), not significant]. Animals were found dead in their cages and their brains were not analysed. Nine additional animals (three in group I, four in group II, two in group III) were eliminated because respiratory arrests developed during light ether anaesthesia. Brain analysis was not performed in these rats. Two rats (one in group I and one in group II) were not included in the study because of a too small maximum daily SNa+ increment (maximum daily SNa+ increase observed on 1 of the 2 days of the correction period = ΔSNa+ < 10 mmol/l and were found to be free of brain lesions. Despite an identical treatment regimen in each group during the 2 days of the correction period, rats presented successive daily ΔSNa+ of unequal magnitude. The majority achieved on 1 of the 2 days a large ΔSNa+ (ΔSNa+) and on the other day a significantly lower ΔSNa+ (ΔSNa+, P < 0.001) (see Table 1). One of our aims was to determine the maximum daily SNa+ increment tolerated by the brain (see below). A preliminary analysis of the individual data demonstrated that the first brain lesions appeared at an MAxSNa+ > 20 mmol/l. In attempting to establish the toxicity of a daily MAxSNa+ over this limit, an additional deleterious effect of ΔSNa+ during the other day (ΔSNa+) of the correction has to be avoided.

Table 1: SNa+, ΔSNa+ and HS for the three groups with one of the 2 days a maximum individual daily ΔSNa+ > 20 ≤ 30 mmol/l (MAxSNa+) and the other day a ΔSNa+ < 20 mmol/l (mΔSNa+) during the 48 h of the correction period

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial SNa+ (mmol/l)</th>
<th>SNa+ at time of hyponatraemia (mmol/l)</th>
<th>MAxSNa+ (mmol/l)</th>
<th>mΔSNa+ (mmol/l)</th>
<th>ΔSNa+ at time of death (mmol/l)</th>
<th>No. of rats with lesions</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>138 ± 0.6 (135–146)</td>
<td>92 ± 1.7 (83–109)</td>
<td>24 ± 0.4 (21–29)</td>
<td>12 ± 0.6 (3–18)</td>
<td>36 ± 0.9 (26–46)</td>
<td>139 ± 0.5 (135–147)</td>
<td>9/23 (39%)</td>
</tr>
<tr>
<td>Group II</td>
<td>138 ± 0.6 (135–145)</td>
<td>91 ± 1.3 (85–103)</td>
<td>24 ± 0.5 (21–29)</td>
<td>13 ± 0.9 (1–19)</td>
<td>37 ± 1.1 (28–48)</td>
<td>136 ± 0.5 (133–145)</td>
<td>15/23 (65%)</td>
</tr>
<tr>
<td>Group III</td>
<td>139 ± 0.4 (136–145)</td>
<td>97 ± 1 (88–109)</td>
<td>24 ± 0.5 (21–30)</td>
<td>13 ± 0.9 (0–19)</td>
<td>37 ± 0.9 (29–45)</td>
<td>140 ± 0.9 (130–150)</td>
<td>18/25 (72%)</td>
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</table>
Indeed, if the threshold of tolerance is exceeded a second time it becomes impossible to determine which ΔSNa⁺ has induced the brain damage. The HS also becomes difficult to interpret as it will not necessarily reflect a simple additional effect of both toxic daily ΔSNa⁺. Therefore, we have excluded rats which have exhibited also on the other day a ΔSNa⁺ (mean ΔSNa⁺ 30 mmol/l and mean ΔSNa⁺ 25 mmol/l) and were severely injured (HS = 3–8) (see below). Fourteen animals (six in group I, four in group II, four in group III) were also excluded because they were overcorrected and their brains were not analysed. The eight overcorrected rats treated with hypertonic saline presented a very large ΔSNa⁺ on 1 of the 2 days of the correction period (ΔSNa⁺ 30–40 mmol/l). All of them were severely symptomatic. Two of the six rats treated with urea were symptomatic and had high ΔSNa⁺ (29 and 32 mmol/l) and SNa⁺ on the other day (ΔSNa⁺ > 20 mmol/l). The four other rats had a ΔSNa⁺ of 17–20 mmol/l and were all asymptomatic, regaining weight between day 6 and day 8.

Clinical and histological data

Most of the rats that developed severe hyponatraemia became apathic. When rats with brain damage demonstrated clinical symptoms, during or after the treatment of their hyponatraemia, they became extremely hyperactive and hyperirritable, running and jumping all over the cage in response to stimulus. This was very often associated with spasticity of the extremities and an ataxic gait. Independently of the treatment used, after losing weight between days 1 and 6 (−27 ± 6 %), rats which exhibited brain damage continued to lose weight (−9 ± 4 %, P < 0.001). Rats which were free of brain lesions regained some weight between day 6 of the correction period and day 8 (+12 ± 4 %, P < 0.001). Pathological changes in the rats killed at day 8 of the experiments were similar to those previously described [2, 5, 20, 22]. None of the rats with a normal brain displayed symptoms. In the group of rats with a HS of 1 (n = 9) only one rat had symptoms (11 %). This incidence increases progressively with the development of more severe HS; HS of 2 = 36 % (4/11); 3 = 67 % (6/9); 4 = 67 % (8/12); HS > 5 = 94 % (15/16).

Comparison between treatment with hypertonic saline in boluses (group II) or in divided doses (group III)

It must be noted that in each further analysis made between various groups of rats their respective SNa⁺ values are comparable. Fig. 1 shows the evolution of SNa⁺ over the first 8 h after an injection of hypertonic saline in boluses or in divided doses. The rate of increase in SNa⁺ was different with both methods. We observed after 1 h a significant rise in SNa⁺ (ΔSNa⁺ 19 ± 3.3 mmol/l) in group II compared with the slight increment (ΔSNa⁺ 2 ± 1.2 mmol/l) in group III (P < 0.001). The slope of the increase in SNa⁺ in group III indicates a more progressive correction with a mean rate of 1.1 ± 0.1 mmol h⁻¹ l⁻¹ (range 0.8–2 mmol h⁻¹ l⁻¹) for the first 8 h. In group II, after the initial peak of SNa⁺ in the first hour, the subsequent change in natraemia was slight but SNa⁺ was still significantly higher after 8 h as compared with group III (P < 0.001). Despite the major difference in the kinetics of SNa⁺, correction described above, both modes of administration of hypertonic saline appear to be equally toxic for the brain. The level of maximum daily ΔSNa⁺ above which neurological damage appeared was similar in group II (n = 46) and group III (n = 39) (see Fig. 2). For 95 % of the rats the lesions appeared when the daily ΔSNa⁺ was > 20 mmol/l. However, in both groups we could identify in the range of ΔSNa⁺ > 20 < 23 mmol/l a 'transition zone' above which nearly all rats presented cerebral damage. In this transition zone, a lower number of rats were injured (33 % in group II, 42 % in group III) with less severe mean HS (0.9 ± 0.4 in group II, 1.7 ± 0.7 in group III, not significant) as compared with rats which presented a ΔSNa⁺ > 23 mmol/l (respectively 100 % and 86 %). Both modes of administration of hypertonic saline were equally toxic for the brain whichever range of ΔSNa⁺ was analysed (see Fig. 3).

The results in Fig. 3 also indicate that the severity of brain lesions increases gradually with the magnitude of the ΔSNa⁺ achieved by the rats (r = 0.77 and r = 0.63, P < 0.001 for group II and III). Table 1 gives data in the ΔSNa⁺ > 20 < 30 mmol/l range, which contains nearly all the animals with brain lesions obtained in our experiments. The severity of the lesions was not influenced by the day (first or second day) on which the toxic ΔSNa⁺
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Fig. 2. Relationship between \( \Delta S_{\text{Na}^+} \) and levels of brain damage (HS) after treatment with urea (group I, \( n = 51 \), a), hypertonic NaCl given as one bolus injection per day (group II, \( n = 46 \), b) and hypertonic NaCl of the same total dose as group II given as six injections per day (group III, \( n = 39 \), c). Each point represents individual data (HS and \( \Delta S_{\text{Na}^+} \)) for one rat in each group. The \( \Delta S_{\text{Na}^+} \) achieved during the other day (m\( \Delta S_{\text{Na}^+} \)) was <20 mmol/l for all the rats.

was reached (for instance in group II, mean HS = 1.6 ± 0.4 in rats (\( n = 11 \)) with \( \Delta S_{\text{Na}^+} = 24 ± 1 \) mmol/l occurring on the first day and HS = 2 ± 0.5 \( \Delta S_{\text{Na}^+} = 24 ± 0.6 \) mmol/l, \( n = 17 \) for the second day; not significant).

One rat in group III had achieved its \( \Delta S_{\text{Na}^+} \) (\( = 25 \) mmol/l) on the third day of the correction after undergoing 2 days of small \( \Delta S_{\text{Na}^+} \) (day 1 \( \Delta S_{\text{Na}^+} = 8 \) mmol/l; day 2 \( \Delta S_{\text{Na}^+} = 9 \) mmol/l) and had brain injuries (HS = 3).

Rats with brain damage despite a persistent hyponatraemia (\( S_{\text{Na}^+} < 129 \) mmol/l) and rats without brain damage despite a normonatraemia after 48 h

After the 2 days of the correction period, 10 animals (three in group I; two in group II and five in group III) had pronounced brain lesions (HS 2.3 ± 0.3) and were still hyponatraemic (\( S_{\text{Na}^+} = 126 ± 0.8 \) mmol/l, range 120–128 mmol/l). It must be emphasized that, for all of them, the \( \Delta S_{\text{Na}^+} \) was, however, >20 mmol/l (25 ± 0.6 mmol/l, range 22–28 mmol/l). Ten rats (three in group I, four in group II and three in group III) had achieved a normonatraemia by 48 h of the correction period after an \( S_{\text{Na}^+} \) increase >30 mmol/l (36 ± 1.2 mmol/l 48 h\(^{-1} \) \, 1\(^{-1} \), range 31–42 mmol/l 48 h\(^{-1} \) \, 1\(^{-1} \) and did not develop brain lesions. Each had a \( \Delta S_{\text{Na}^+} < 23 \) mmol/l and a m\( \Delta S_{\text{Na}^+} \) <19 mmol/l.

Comparison between treatment with urea (group I) or with hypertonic saline (group II and group III)

Fig. 1 demonstrates that the kinetics of \( S_{\text{Na}^+} \) correction in the rats treated with urea and those given hypertonic saline in divided doses are similar. Table 1 shows that the rats in group I (\( n = 51 \)) present a \( S_{\text{Na}^+} \) evolution over 48 h comparable with that of groups II and III. At the time of hyponatraemia, group I had a lower \( S_{\text{Na}^+} \) than group III (92 ± 1.7 compared with 97 ± 1 mmol/l, \( P<0.01 \)). The threshold of appearance of the first brain lesions is represented by a \( \Delta S_{\text{Na}^+} \) of 20 mmol/l, which is not different from that in the other groups. As with hypertonic saline, HS increased with the magnitude of the \( \Delta S_{\text{Na}^+} \) (\( r = 0.63 \), \( P<0.001 \)). Comparative analysis of urea treatment against both modes of hypertonic saline administration demonstrates the lower toxicity obtained in the rats treated with urea (see Table 1 and Fig. 3). As shown in Fig. 3, rats treated with urea present a significantly lower HS than rats in group II and group III except in the range >20 <25 mmol/l, where only a trend for a less toxic effect of urea was observed when compared with group II. However, this range of >20 <25 mmol/l includes the 'transition zone' previously described. This difference was strengthened if we excluded rats situated in this 'transition zone'. In the \( \Delta S_{\text{Na}^+} \) range >23 <30 mmol/l, the mean \( \Delta S_{\text{Na}^+} \) was similar in the three groups (26 ± 0.5 for group III, not significant).

Less severe histological lesions were present in the group treated with urea (HS 1.2 ± 0.3 for group I; 3.3 ± 0.3 for group II, \( P<0.005 \); 3.6 ± 0.5 for group III, \( P<0.005 \)). A lower number of rats (47%) were also
damaged in group I \( P<0.01 \) compared with group II (100\%) and compared with group III (89\%) \( P<0.02 \). If we compare rats which have achieved their \( \Delta S_{Na^+} \), on the same day (second day) in group I and group II, for example, the lower toxicity in rats of group I is still observed: group I \( (n = 15) \), mean \( \Delta S_{Na^+} = 25 \pm 0.5 \) and HS = 0.6 \pm 0.3; group II \( (n = 11) \), \( \Delta S_{Na^+} = 25 \pm 0.6 \) and HS = 2.9 \pm 0.4, \( P<0.001 \), range \( > 23 \leq 30 \) mmol/l). In four additional rats made hyponatraemic by the same procedure, blood urea concentrations and \( S_{Na^+} \), were measured 2 h after one intraperitoneal injection of the same dose of urea in group I: initial \( S_{Na^+} \): 107 \pm 1.6 mmol/l, blood urea 4.5 \pm 0.25 mmol/l. After 2 h \( S_{Na^+} \) was 108 \pm 2 mmol/l and blood urea was 22.1 \pm 3 mmol/l.

**Influence of the severity of the hyponatraemia**

The purpose was to determine if the severity of the hyponatraemia before correction could predispose to the risk of subsequent ODS. Therefore, in the groups of rats treated with hypertonic saline (groups II and III) two subgroups were compared: the first subgroup (A) includes rats which presented a decrease in \( S_{Na^+} \), (difference between initial \( S_{Na^+} \) and \( S_{Na^+} \) on day 4) in the range 41–50 mmol/l \( (46 \pm 0.4 \) mmol/l, \( n = 25) \) and the second subgroup (B) includes rats with a decrease in the range 30–40 mmol/l \( (36 \pm 0.6 \) mmol/l, \( n = 17) \). Both subgroups have achieved a similar \( \Delta S_{Na^+} \) \( (25 \pm 0.6 \) for subgroup A and 24 \pm 0.7 for subgroup B). No difference was observed in the HS of both subgroups with hyponatraemia of different degrees of severity \( (2.7 \pm 0.4 \) for subgroup A and 2.7 \pm 0.6 for subgroup B).

**DISCUSSION**

Within each of the three groups, the treatment regimen was identical during the 2 consecutive days of the correction. Nevertheless, most of the rats presented a large \( \Delta S_{Na^+} \), (\( \Delta S_{Na^+} \)) on 1 of the 2 days and a low \( \Delta S_{Na^+} \), (\( \Delta S_{Na^+} \)) on the other day, without any predictive clues. The mechanisms responsible for the variability of the individual response to therapy and consequently, the difficulty in controlling the extent of the correction, are not fully understood [6, 19, 26]. Brain lesions developed after the treatment of hyponatraemia by either hypertonic saline or urea demonstrated symmetrical extrapontine distribution similar to those previously described [2, 5, 20, 22]. Interestingly, we have observed a direct relationship between the severity of the histological damage and the incidence of the clinical symptoms. Although 60\% of the injured rats in the three groups presented clinical symptoms, only 11\% of the animals with the lower grade of HS \( (HS = 1) \) were symptomatic. When the HS was \( >5 \), 94\% of the animals displayed symptoms.

Our results indicate that the correction of chronic severe hyponatraemia with an absolute increment in \( S_{Na^+} \) > 20 mmol/l induces significant brain damage in rats treated with hypertonic saline. Indeed, for 95\% of the injured rats in groups II and III, the first brain lesions appeared when they were submitted to a \( \Delta S_{Na^+} \) of 20 mmol/l on 1 of the 2 days of the correction period. In a recent study, Ayus et al. [8] concluded that in rats treated with hypertonic saline, brain damage results from a correction by an absolute increment in \( S_{Na^+} \), of at least > 25 mmol/l in the first 24 h. Below this limit, 100\% of the animals survived. We have demonstrated here that significant brain lesions could occur when the \( S_{Na^+} \) was increased with a \( \Delta S_{Na^+} \), between 20 and 25 mmol/l. Beyond the threshold of 20 mmol day\(^{-1}\) \( l^{-1} \), the correction of \( S_{Na^+} \), with a more important \( \Delta S_{Na^+} \), was progressively associated with an increased incidence of more severe brain damage. However, the \( \Delta S_{Na^+} \) range \( > 20 < 23 \) mmol/l can be considered as a ‘transition zone’ where 33–42\% of the rats treated with hypertonic saline were injured, as compared with 89–100\% in the \( \Delta S_{Na^+} \) range \( >23 \) mmol/l. The concept of a ‘transition zone’ could explain the absence of brain lesions reported by Ayus et al. [8] for rats corrected with a daily \( \Delta S_{Na^+} \) of 20–25 mmol/l. Experimental data on the tolerance of rat brain to increases in \( S_{Na^+} \), during the first 48 h of the treatment are lacking [2, 4, 5, 8]. The outcome of the treatment was generally evaluated by considering the kinetics of \( S_{Na^+} \) during the first 24 h of the correction. With support from clinical data, it is recommended that hyponatraemia be corrected without exceeding a \( \Delta S_{Na^+} \) of 25 mmol/l in 48 h and also avoiding reaching normonatraemia [14, 18].
In fact, our results indicate that rats could tolerate a ΔS Na⁺, largely above 25 mmol 48 h⁻¹ l⁻¹ (31–42 mmol/l), even up to a normonatraemic level, provided that the ΔΔS Na⁺ was not higher than the maximum daily tolerated threshold or was included in the 'transition zone'. Two consecutive daily ΔS Na⁺, close or equivalent to the limit of 20 mmol/l could probably be safely achieved. On the other hand, the consequences of an overly large increment in S Na⁺ are similar whether it arises during the first 24 h or during the second and possibly even during the third day of the correction period.

Our results underline the importance of the absolute increment in S Na⁺, whatever the final level of S Na⁺ or the time necessary to reach the 'critical threshold' of ΔS Na⁺. Indeed, although widely advocated in the guidelines for the management of hyponatraemia [5, 11, 13, 14], our data confirm [6–8] the potential risk of a correction of S Na⁺ to mildly hyponatraemic levels (<129 mmol/l) when the daily ΔS Na⁺ is >20 mmol/l. Ten rats developed significantly brain injuries despite a final S Na⁺ between 120 and 129 mmol/l. On the other hand, after boluses injections of hypertonic saline the brain is rapidly submitted to an osmotic stress (S Na⁺ of 19 mmol/l in the first hour) sufficient to promote significant water shifts across the blood–brain barrier, a gradient generally evaluated around 25–30 mosmol/kg H₂O [27]. Brain volume is a function of its tissue content in intracellular active solutes [28] which decreases during hyponatraemia [17, 28, 29]. Consequently, the brain becomes more susceptible to dehydration during the subsequent rise in S Na⁺ because of the slow recovery of these intracellular osmoles [7, 30]. This critical factor does not seem to play a role during the correction period studied here. Indeed, our results indicate that both gradual (NaCl in divided doses, group III) and rapid (NaCl in boluses, group II) S Na⁺ increases are equally toxic when the ΔS Na⁺ exceeds the tolerated limit of 20 mmol day⁻¹ l⁻¹. This is consistent with the concept of tolerance to a 'rapid increase' proposed by Ayus et al. [10, 18]. From our results it appears that the use of only the mean values of S Na⁺ or ΔS Na⁺ is not an appropriate method to evaluate the problem of the correction of hyponatraemia. Provision of the individual data is essential to the interpretation of the results of the experiments. In the conditions of severity and duration (3 days) of the hyponatraemia studied here, our results do not support the hypothesis that the severity of the pre-existing hyponatraemia decreases the tolerance of the S Na⁺ increase. This study confirms that the susceptibility to the neurological complications of treatment is obviously lower when S Na⁺ is corrected with urea. Indeed, we have observed a lower incidence of injured rats (39% as compared with 65% and 72%) with a significantly lower grade of brain damage in group I compared with groups II and III (see Table 1).

The difference is particularly significant when rats were submitted to a large ΔΔS Na⁺ (range >23 ≤30 mmol/l), which induced severe brain lesions when hypertonic saline was administered (HS: P<0.005). In our previous study [20] the difference between urea and hypertonic saline seemed to be more obvious in that no lesions were observed in the rats treated with urea (n = 8), whereas 100% were damaged in the group receiving hypertonic saline (n = 7). This can now be explained when we analyse the individual ΔΔS Na⁺ achieved by the rats in each group of this previous work, showing that all the ΔΔS Na⁺ values were >20 mmol/l (range 21–36 mmol/l) on 1 of the 2 days of the correction period in the group treated with hypertonic saline. In the group treated with urea, only two rats had a ΔΔS Na⁺ <20 mmol/l (17 and 18 mmol/l), the others achieving a ΔΔS Na⁺ of between 22 and 38 mmol/l without brain injury. It is important to note that the definite limit of tolerance of the brain to a daily ΔΔS Na⁺ defined here in rats cannot be extended to man.

The mechanisms explaining the protective role of urea are only hypothetical and are worthy of further investigation. The better tolerance of brain to S Na⁺ correction could not be attributed to a more gradual rise in S Na⁺ for the rats treated with urea as discussed above. Urea, which penetrates the brain progressively (equilibration with serum concentrations in 4–10 h) [31], could possibly prevent a secondary excessive dehydration due to the rise in S Na⁺ by providing intracellular osmoles to brain cells depleted in electrolytes by 70%, the other solutes lost being amino acids among others [17]. Interestingly, the brain is probably also submitted to a significant osmotic gradient early after the intraperitoneal injection of urea and largely before that a significant ΔΔS Na⁺ is achieved as S Na⁺ is slowly corrected. This early osmotic stress for the brain could trigger the mechanisms of solute recovery and thereby could prepare brain to support the subsequent rise in S Na⁺. After rapid correction (9 h) of chronic hyponatraemia in mice by hypertonic saline, the brain amino acid content remained uncorrected (two-thirds of the control value) [30]. It has been shown that exogenous urea could be a potential source of nitrogen for amino acids synthesis in malnourished human subjects [32]. We also know that CPM has been frequently described in alcoholic and malnourished patients with debilitating diseases. Therefore, this represents another possible mechanism by which urea could decrease the susceptibility of brain to an osmotic stress.

Finally, some clinical arguments exist for the advantage of an osmotic diuresis induced by urea as compared with the use of hypertonic saline [33]. We now have a great deal of clinical experience of the use of urea in the treatment of hyponatraemia of various origins [23, 34–39] and, like other workers [26, 40], we have not observed neurological complications despite a sometimes rapid S Na⁺ rise of important magnitude [23]. These results appear to support the undertaking of a clinical prospective study.

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