Effects of Urea and Other Dehydrating Agents Upon Dog Brain

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The successful use of urea in neurosurgery for the reduction of brain volume has led to many studies of the effects of hypertonic solutions of this compound upon the physiology of the intact animal. Few investigations, however, have been made of the biochemical changes which occur in the brain during and immediately following the infusion of urea. It has been considered by many authors, despite data to the contrary,1,15,16,20 that urea does not penetrate the brain and that its effects are essentially produced by its hypertonicity. The following studies were undertaken to determine the effects of urea upon the chemical constituents of the brain under conditions simulating human neurosurgery and at a time when the brain was beginning to show maximum shrinkage, i.e. 45 minutes after the beginning of the infusion of urea.

Methods

Mongrel dogs anesthetized with intravenous pentobarbital were given 1 gm./kg. of urea (30% solution) in 10 per cent invert sugar (Urevert©) over a period of 30 min. by intravenous infusion. The animals were prepared by placing polyethylene catheters in a branch of the femoral artery to facilitate the drawing of arterial blood samples and in the sagittal sinus of the brain for the removal of cerebral venous blood. A spinal needle was placed in the cisterna magna. Samples were taken before the infusion was started, 15 min. after urea had been started and 15 min. after the infusion of urea had been stopped; that is 45 min. from the beginning of the infusion of urea. The arterial and venous blood samples were drawn simultaneously and the cerebrospinal fluid samples as quickly as possible thereafter. As soon as the last sample had been drawn the urea was washed with deionized water, dried with suction, opened and a vertical core of brain tissue extending from the cortex above to the base of the midbrain below was removed. The sample was taken immediately caudad to the cruciate sulcus and included most of the postcrucial gyrus, thus avoiding the large dorsal cerebral veins. Care was taken to remove the meninges and any visible blood. The sample from the left side of the brain was divided longitudinally, one-half being used for determinations of moisture by the conventional method of drying in a vacuum, the other half being frozen in dry ice and acetone for determinations of content of urea. The symmetrical sample from the right side was removed in a similar manner, divided in half longitudinally and frozen for the determination of sodium, potassium and chloride. The brain samples, therefore, contained similar quantities of gray and white matter. Samples of gray matter from the caudate nucleus and of white matter from the corpus callosum were also removed for analysis of urea. The blood determinations of urea* and glucose were carried out by the methods of Karr1 and Nelson16 respectively. The flame photometer method was used for the determination of sodium, potassium and a modification of Whitehorn's5 method for the determination of chloride. The urea present in the brain tissue† was determined by the method of Engel and Engel19 as modified by Greenberg.5 Standard statistical methods were used for the calculation of standard deviation and t (test for significance). The percentage of loss of water was calculated according to the formula given by Van Harreveld et al.21

For comparison with the effects of urea, the biochemical changes occurring in the brain were determined after the infusion of 50 per cent solution of glucose or of a 20 per cent solution of mannitol.21 The glucose was infused at an average rate of 3.3 cc./min. for 30 min.; the mannitol, 0.89 gm./kg. in the same length of time. In order to separate the effects of the urea from those of

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FIG. 1. Brain arteriovenous differences in blood urea during the administration of urea and of invert sugar. Cerebrospinal-fluid concentrations of urea under the same conditions. Arterial blood from the femoral artery; venous blood from the sagittal sinus.

The infusion of urea resulted in a high blood level of urea in 15 min. There was an arteriovenous difference in urea between the arterial blood and blood drawn from the sagittal sinus. Urea appeared in the spinal fluid more slowly and only began to approach the concentration of the cerebral venous blood about 45 min. after the urea had been started (Fig. 1). During the time that the urea was being infused there also appeared to be an increase in the amount of glucose removed from the blood in that the arteriovenous differences were greater during the infusion of the urea than before the infusion in a similar group of control animals or in animals receiving invert sugar alone (Fig. 2). The data on the loss of brain water showed that urea given in invert sugar was the most efficient way of dehydrating the brain. Next in order of efficiency were 50 per cent glucose, 20 per cent mannitol, urea in saline, saline alone and 10 per cent invert sugar (Table 1). The differences in percentage of water loss were not great in the entire series.

Although all these agents removed water from the brain, their effects upon the electrolyte content of the brain were quite different. If we consider the electrolyte data on the basis of wet weight (Table 1), we find that urea when given in invert sugar had a slight tendency to increase the level of sodium without

FIG. 2. Brain arteriovenous differences in blood sugar during the administration of urea and of invert sugar. Arterial blood from the femoral artery; venous blood from the sagittal sinus.
Changes in electrolytes and water in brain during dehydration (mg.% wet weight)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Na Level</th>
<th>SD</th>
<th>K Level</th>
<th>SD</th>
<th>Cl Level</th>
<th>SD</th>
<th>Water Per Cent</th>
<th>SD</th>
<th>Water Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>152.0</td>
<td>25.6</td>
<td>340.7</td>
<td>31.6</td>
<td>162.3</td>
<td>7.5</td>
<td>76.8</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>10% invert sugar</td>
<td>13</td>
<td>211.9†</td>
<td>15.5</td>
<td>379.7</td>
<td>78.2</td>
<td>165.1</td>
<td>10.3</td>
<td>75.7</td>
<td>2.3</td>
<td>4.53</td>
</tr>
<tr>
<td>Urea*</td>
<td>16</td>
<td>180.7†</td>
<td>19.3</td>
<td>362.3</td>
<td>50.2</td>
<td>164.1</td>
<td>8.1</td>
<td>74.8†</td>
<td>1.8</td>
<td>7.94</td>
</tr>
<tr>
<td>Urea**</td>
<td>12</td>
<td>108.7†</td>
<td>15.7</td>
<td>368.5</td>
<td>60.5</td>
<td>188.6‡</td>
<td>36.0</td>
<td>75.4‡</td>
<td>1.3</td>
<td>5.69</td>
</tr>
<tr>
<td>50% glucose</td>
<td>5</td>
<td>200.8‡</td>
<td>19.5</td>
<td>469.2‡</td>
<td>22.7</td>
<td>157.2</td>
<td>5.6</td>
<td>75.0‡</td>
<td>0.5</td>
<td>7.20</td>
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<tr>
<td>20% mannitol</td>
<td>6</td>
<td>112.4‡</td>
<td>12.7</td>
<td>332.6</td>
<td>90.0</td>
<td>167.0</td>
<td>8.6</td>
<td>75.3†</td>
<td>1.6</td>
<td>6.07</td>
</tr>
<tr>
<td>Saline</td>
<td>6</td>
<td>134.5‡</td>
<td>5.2</td>
<td>355.7</td>
<td>31.6</td>
<td>159.8</td>
<td>4.8</td>
<td>75.5‡</td>
<td>0.8</td>
<td>5.31</td>
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</tbody>
</table>

* 30% urea in 10% invert sugar.
** 30% urea in physiological saline.
† P=0.05.
‡ P=0.01.
§ Compared to the control values.
SD = standard deviation.

affecting the levels of potassium and chloride. The animals given 50 per cent glucose, on the other hand, showed a highly significant increase in levels of both sodium and potassium of the tissue with very little change in the level of chloride. With mannitol or urea in saline the animals showed a significant decrease in the amount of sodium in the brain with little alteration of either chloride or potassium. When saline alone was given there was a small fall in sodium and a rise in the level of chloride. The animals receiving invert sugar had an increase in cerebral sodium with little change of the other ions. Essentially the same relations appear in the data calculated as mEq. on a basis of dry weight (Table 2).

Determinations of urea were made on mixed gray and white matter, from 9 dogs in each of the groups receiving urea in invert

TABLE 2
Changes in electrolytes and water in brain during dehydration (mM./kg. dry weight)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Na Level</th>
<th>SD</th>
<th>K Level</th>
<th>SD</th>
<th>Cl Level</th>
<th>SD</th>
<th>Water Per Cent</th>
<th>SD</th>
<th>Water Per Cent</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>280.6</td>
<td>39.2</td>
<td>373.4</td>
<td>30.8</td>
<td>197.5</td>
<td>10.7</td>
<td>76.8</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>10% invert sugar</td>
<td>13</td>
<td>377.7‡</td>
<td>29.2</td>
<td>399.6</td>
<td>29.8</td>
<td>191.4</td>
<td>10.6</td>
<td>75.7</td>
<td>2.3</td>
<td>4.53</td>
</tr>
<tr>
<td>Urea*</td>
<td>16</td>
<td>308.3</td>
<td>39.6</td>
<td>364.4</td>
<td>17.8</td>
<td>184.1†</td>
<td>16.9</td>
<td>74.8‡</td>
<td>1.8</td>
<td>7.94</td>
</tr>
<tr>
<td>Urea**</td>
<td>12</td>
<td>191.8‡</td>
<td>25.8</td>
<td>384.7</td>
<td>58.1</td>
<td>216.3†</td>
<td>28.8</td>
<td>75.4‡</td>
<td>1.3</td>
<td>5.69</td>
</tr>
<tr>
<td>50% glucose</td>
<td>5</td>
<td>349.3‡</td>
<td>26.5</td>
<td>480.8†</td>
<td>16.2</td>
<td>177.4‡</td>
<td>6.3</td>
<td>75.0‡</td>
<td>0.5</td>
<td>7.20</td>
</tr>
<tr>
<td>20% mannitol</td>
<td>6</td>
<td>199.2‡</td>
<td>30.5</td>
<td>324.2†</td>
<td>33.7</td>
<td>191.0†</td>
<td>15.7</td>
<td>75.3†</td>
<td>1.6</td>
<td>6.07</td>
</tr>
<tr>
<td>Saline</td>
<td>6</td>
<td>220.2‡</td>
<td>15.6</td>
<td>373.0</td>
<td>42.8</td>
<td>183.2†</td>
<td>10.5</td>
<td>75.5‡</td>
<td>0.8</td>
<td>5.31</td>
</tr>
</tbody>
</table>

* 30% urea in 10% invert sugar.
** 30% urea in physiological saline.
† P=0.05.
‡ P=0.01.
§ Compared to the control values.
SD = standard deviation.
sugar, invert sugar alone, and the control. The average contents of urea were 120.1 mg. per cent, 15.0 mg. per cent and 19.1 mg. per cent respectively. Separate samples of gray and white matter were analyzed in 5 animals from each group. Gray matter showed 21.7 mg. per cent for the control animals; 19.9 mg. per cent for the invert sugar animals; and 19.2 mg. per cent for the urea animals. White matter tended to show a lower content of urea, being 18.2 mg. per cent, 17.7 mg. per cent and 12.6 mg. per cent respectively.

Discussion

Our experiments have been designed to reproduce as nearly as possible the conditions occurring in the patient receiving urea in preparation for neurosurgical procedures. For this reason the total dose of urea has been kept low, the infusion was carried out at a slow rate and the samples were taken at uniform times. In the few dogs tested, cerebrospinal-fluid pressure fell so that 15 min. after the urea had been stopped it was only slightly more than half the original value; for example, to 65 mm. H₂O as compared to a control value of 117 mm. H₂O. At this time, judged by visual observation and by ease of manipulation, the brain showed marked shrinkage. The comparisons of animals given urea at the same rate are important since Loehning and his colleagues have shown that the time at which the brain returns to its initial size is directly related to the rate at which the urea is administered; the faster the rate the more quickly the brain returns to normal size. The degree to which the infusion of urea raised the osmolarity of the blood can be seen by a comparison of blood from control animals and those receiving urea, sampled 30 min. after infusion of urea was completed. The osmolarity of arterial blood expressed as mOsm./l was 310.9 (5 animals) and 330.8 (3 animals) respectively. The figures for venous blood were slightly higher, 313.8 and 332.9 respectively.

Our data undoubtedly were obtained during the period when dynamic changes were occurring in the brain. It is hoped that we shall be able at a later time to present evidence on events in the brain after it has attained an equilibrium. The design of these studies makes it impossible to compare our data with investigations in which urea was given in large doses or in which brains were removed at varying times. We feel that such studies while of theoretical interest have little bearing on the clinical use of urea.

At the level of dosage and the speed of infusion used, little if any effect was observed in heart rate, blood pressure or electrocorticogram. The data show clearly that urea enters the brain as has been described by others. In accordance with Schoolar et al. it appears to enter white matter less readily than gray. The increase in arteriovenous difference in glucose has suggested to us that there may be at least a temporary slowing in the blood flow and the data on this parameter will be reported elsewhere. Luse has shown that urea dehydrates the cytoplasm of the oligodendroglia, cells which may serve in the transport of material from blood to brain. If these cells are dehydrated by urea one could expect to find a depressed transport of glucose during the period of dehydration. Our data unfortunately offer nothing to clarify this situation.

Although the individual variations in electrolytes in general are large (see the standard deviations given in the tables) we feel that they reflect the dynamic state of the brains at the time the samples were taken as well as differences in the proportions of gray and white matter in the sample, rather than technical difficulties. The values for the control animals compare well with data in the literature. In spite of the variations certain patterns are evident. It is important in discussing these changes to keep in mind that if an electrolyte remains at the same concentration in the dehydrated brain as in the control we must assume that the electrolyte has moved from the brain with the water thus maintaining the same concentration in brain. A constant value, therefore, means a movement from the brain with the water; an
increase, that water moved without electrolyte, and a decrease that the water leaving the brain contained more than the usual cerebral level of the electrolyte. To explain the phenomena observed we have assumed that glia contain high concentrations of sodium as suggested by Katzman and by Koch et al. and may serve as repositories for sodium, chloride and water as portrayed by Gerschenfeld et al. It is also necessary to assume that movements of urea and chloride in some fashion balance each other as do also glucose or invert sugar and sodium or potassium. On this basis invert sugar might have produced its dehydrating effect by removing water and chloride from the glia leaving behind a higher concentration of sodium, and by removing water and potassium from neurons. The movement of invert sugar into the brain, therefore, would appear to inhibit or balance that of sodium, but not that of potassium. Urea dissolved in invert sugar dehydrated the brain by removing water complete with its usual cerebral content of sodium, potassium and chloride so that the concentration of these substances on the basis of a wet weight was unchanged. Bradbury and Coxon felt that electrolytes moved with the water from brain when urea in invert sugar was used as the dehydrating agent. Our data would tend to support this view. On the other hand, if urea is given in saline, potassium is withdrawn from the brain with the water, but sodium is removed in excess and chloride remains in the tissue. These changes suggest some competition between the movements of urea and of chloride since if saline is given alone the concentration of chloride is relatively unchanged.

When 50 per cent glucose is used as the dehydrating agent water appears to move out of the brain taking chloride with it, but leaving behind sodium and potassium so that the concentrations of these two cations increase. It might be reasoned then that 50 per cent glucose dehydrated both the glia and the neurons removing only water and chloride from these cells. In this way the movement of glucose could be considered to balance that of sodium and potassium in a similar fashion to the relation between urea and chloride. Van Harreveld et al. found in rabbits that both chloride and sodium were lost from the tissue when an infusion of 50 per cent glucose was continued until death occurred. Our animals showed much less dehydration, 7.2 per cent as compared to 25.3 per cent, and only a slight loss of chloride (compare figures for dry and wet weight). Our data suggest that under these circumstances chloride may move out of the cells first, followed by sodium if the dehydration is continued long enough. Elucidations of these processes will require more detailed and sophisticated studies.

Our data unfortunately contribute little additional information on the mechanism of action of urea or on the fate of urea in the brain. It is difficult to believe, even though no urease has been found in brain, that such a metabolically active substance as urea remains unmetabolized. Reed and Woodbury have described a slow and fast compartment of penetration of urea in rat brain and have not been able to demonstrate any metabolism of C-labeled urea. Bradbury and Coxon believed that a difference in species exists in the penetration of urea into the brain, cats and rabbits being more resistant than dogs. If such differences of species exist, the data obtained on experimental animals may bear little relation to the situation in man.

Rosomoff argued on the basis of his experiments with dogs given large doses of urea that the water leaving the brain merely goes to increase the volume of cerebrospinal fluid, the total volume of the cranial cavity remaining constant. The Monro-Kellie doctrine in its statement of constant total cranial volume presupposes a constant intracranial pressure. The rapid readjustment in the compartments of cranial fluid upon changing the volume of one of them has as, at least part of, its function the maintenance of constant pressure.

It is difficult, therefore, to postulate any basis for the marked decrease of cerebrospinal-fluid pressure seen upon infusion of hypertonic solution of urea if the total cranial volume remains unchanged. If it is as-
assumed that formation of cerebrospinal fluid remains constant, Reed and Woodbury visualized that the blood containing urea removes water from the brain and transiently visualized that the blood containing urea resumed that formation of cerebrospinal fluid and the brain vascular compartment. The cerebrospinal-fluid pressure does not return to normal until the rate at which water is being withdrawn is less than the rate of increase of volume of cerebrospinal fluid and the brain vascular compartment. The slow rate of return of vascular, brain and cerebrospinal-fluid volumes to normal permits cerebrospinal-fluid pressure to remain constant.

Summary

The brain actively removes urea from the blood during infusion of urea (1 gm./kg. of urea in 10 per cent invert sugar). During this time the arteriovenous difference of glucose in the brain is larger in animals receiving urea than in the control group.

The changes in water and electrolytes of brain tissue with various dehydrating agents used for neurosurgery have been explored. Urea (1 gm./kg. of body weight) in invert sugar given in a 30-min. period produced the greatest dehydration and the least changes in electrolytes. Fifty per cent glucose, which reduced the brain volume almost as well as the urea, resulted in a marked rise of sodium and potassium in the brain. The results illustrate the dynamic state of the brain electrolytes at the time of maximum shrinkage.

References