STUDIES ON THE INCREASED POTASSIUM CONCENTRATION OF PLASMA PRODUCED BY A HYPERTONIC SOLUTION OF LOW POTENT SUBSTANCES INTRAPERITONEALLY ADMINISTERED TO RATS.

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Abstract.....Intracranial hemorrhage was induced in rats by low potent substances, glucose, NaCl and Na₂SO₄, intraperitoneally injected in enormous amounts, that is, 2800 mOsmol/L (J. Toxicol. Sci. 5, 290, 1980). The present study was undertaken to examine a mechanism of the above-mentioned phenomenon in detail from the aspects of the disturbance of water-electrolyte balances and the change in blood osmolality. After administration of hypertonic solutions, blood and abdominal fluid were obtained at intervals of 5 to 15 min and at death. Hypertonic solutions injected intraperitoneally induced rapid exchange in water and solutes across the peritoneum, thus causing an increase in abdominal fluid volume and plasma osmolality. Most interesting was the fact that a marked potassemia was produced and that the value of plasma potassium reached 10 mEq/L at death in all of groups of intraperitoneally injected rats. Thus, it is clear that the intracranial hemorrhage is accompanied by an increase in the plasma concentration of potassium which does not always run parallel with an increase in sodium concentration and osmotic pressure in rat plasma.

Key words: hypertonic solution, intraperitoneal administration, hyperpotassemia, hyperosmolality, intracranial hemorrhage.

INTRODUCTION

In administration of drugs to a subject, it is required that the osmolality of the drug solution should be equal to that of the blood. In practice, however, it is usually necessary to extremely elevate the drug concentration for the purpose of lessening its

volume, particularly in case of the investigation of acute toxicity on a low potent substance. On such an occasion, a question always arises as to whether the cause of death involves the toxicity of the drug proper or the physical property of the drug solution, that is, hypertonicity.

According to Finberg (1977), a rapid intravenous injection of 1 M NaHCO_3 to acidotic infants has occasionally led to death from intracranial hemorrhage accompanied by convulsions. Recently, Fukuda et al. (1978), in a study on the acute intravenous toxicity of amino acid reported that the most important determinant factor of its toxicity was osmolality of the solution.

Takeuchi et al. (1980) noticed that practically non-toxic drugs such as glucose (non-dissociated), NaCl (completely dissociated) and Na_2SO_4 (incompletely dissociated) brought about intracranial hemorrhage leading to the death of rats when large doses of these drugs were intraperitoneally injected in high concentrations.

The present study was undertaken to examine the mechanism of the above-mentioned phenomenon in detail from aspects of the disturbance of water-electrolyte balance and the change in blood osmolality.

MATERIALS AND METHODS

The hypertonic solutions used for this study were 50% glucose, 8.12% NaCl and 13.16% Na_2SO_4 , the osmolality of which for all was 2800 mOsmol/L. The experimental animals were female Wistar rats weighing about 200 g. Each of these hypertonic solutions was injected into the abdominal cavity at a volume of 3.5 ml/100 g body wt.

After an intraperitoneal administration of hypertonic solution, rats were killed by ether inhalation at 5, 10, 20, 30, 45 and 60 min after the injection, with the ascites then being collected through an approximately 2 cm incision in the middle abdominal portion. When rats died in convulsions, the death time was noted down and the ascites was collected in the same manner. Blood samples were collected through a heparinized tube inserted into the femoral vein.

Plasma was immediately separated by centrifugation. Na and K in the ascites and the plasma, were measured by a flamephotometer (Hitachi 205), Cl was titrated by the method of Schales and Schales (1941), and osmotic pressure was measured by a vapor pressure osmometer (Wescor Inc. 5100B).

RESULTS

All rats subjected to administration of hypertonic solutions showed similar toxic signs irrespective of the drugs used and died within 120 min after injection. Initially, the rats lay down quietly, and showed sequentially the following symptoms: Anxiety, irritability, violent trembling and finally death by respiratory failure. No essential distinction between the toxic symptoms that developed, however, was recognized among glucose, NaCl and Na_2SO_4 .

Increased plasma potassium by hypertonic solution

Table 1. The average death time in rats injected intraperitoneally with various hypertonic solutions: In Exam. 1, the rats were kept free after injection, in Exam. 2, the rats were subjected to successive collections of blood at intervals of 5 to 15 min. Data are shown as Mean±S. E. (n). ***: Significant difference ($p<0.001$).

	50% Glucose	8.12% NaCl	13.16% Na ₂ SO ₄
Exam. 1	52±3.0 (5)	48±2.6 (5)	40±2.7 (5)
Exam. 2	46±1.7 (7)	87±6.5 (8)***	40±0.4 (7)

Table 1 shows the average death time in three groups of the rats which were intraperitoneally given three types of drugs, respectively: The rats used in Exam 1 were kept free after injection, but the rats in Exam 2 were subjected to successive collections of blood at intervals of 5 to 15 min before and after an injection of drug solution.

There was a significant difference in the death time between glucose and Na₂SO₄ groups ($p<0.05$) in Exam 1. In Exam 2, the death time was markedly prolonged in the NaCl group: [NaCl>glucose ($p<0.001$) and NaCl>Na₂SO₄ ($p<0.001$)] .

1. Time-dependent changes in the fluid component in the abdominal cavity.

Fig. 1. Time-dependent changes in the retention volume of ascites in rats injected intraperitoneally with various hypertonic solutions. Each point shows the Mean±S. E. of five rats. ●—●: glucose, ▲—▲; NaCl, ○—○; Na₂SO₄. *, ** and ***: Significant differences from the value at 5 min ($p<0.05$, $p<0.01$ and $p<0.001$, respectively).

Fig. 2. Time-dependent changes in the sodium concentration of ascites in rats injected intraperitoneally with various hypertonic solutions. See explanation in Fig. 1.

Fig. 1 shows the time-dependent retention of ascites which was one of the characteristic symptoms induced by intraperitoneal injection of the hypertonic solution. The most prominent increase in the volume of ascites was observed in the glucose group. In the NaCl and Na₂SO₄ groups, the volume of ascites increased in a similar way to reach its maximum at the 30 min after injection, and then showed a declining tendency.

Fig. 2 shows the time-dependent changes in the sodium concentration of ascites. The sodium concentration of ascites decreased rapidly within 20 min after injection and then slowly decreased in the NaCl and Na₂SO₄ groups, while it increased slowly but significantly in the glucose group.

As to the disappearance rate of sodium from the abdominal cavity, no significant difference was observed between NaCl and Na₂SO₄ groups, although the time-dependent decrease in the rate was somewhat retarded in the Na₂SO₄ group as seen in Fig. 3.

Fig. 3. Decreasing rates of sodium from ascites in rats injected intraperitoneally with hypertonic solutions, NaCl or Na₂SO₄. Each point shows the Mean $\pm$ S. E. of five rats. $\blacktriangle$ — $\blacktriangle$; NaCl, $\circ$ — $\circ$; Na₂SO₄.

Fig. 4. Time-dependent changes in the chloride concentration of ascites in rats injected intraperitoneally with various hypertonic solutions. See explanation in Fig. 1.

The time-dependent changes in the chloride concentration of ascites ran parallel to the sodium concentration in the NaCl group as seen in Fig. 2 and Fig. 4. The other two groups showed a tendency towards an increase in chloride concentration in ascites, but the increase was not significant.

It was demonstrated that the potassium concentration in ascites increased time-dependently in every group as shown in Fig. 5. At the time of death, the potassium

Increased plasma potassium by hypertonic solution

concentration reached about 10 mEq/L both in the Na_2SO_4 and NaCl groups, and about 8 mEq/L in the glucose group.

Fig. 5. Time-dependent changes in the potassium concentration of ascites in rats injected intraperitoneally with various hypertonic solutions. *: Significant difference from the value at 5 min ($p < 0.05$).

See explanation in Fig. 1.

Fig. 6. Time-dependent changes in the osmolality of plasma in rats injected intraperitoneally with various hypertonic solutions. Each point shows the Mean $\pm$ S. E. of seven rats in the glucose and Na_2SO_4 groups and of eight rats in the NaCl group. ***: Significant difference from the value at 0 min ($p < 0.001$). ●—●; control, ●—●; glucose, ▲—▲; NaCl , ○—○; Na_2SO_4 .

2. Time-dependent changes in osmolality and electrolyte balances in plasma.

In the three groups of rats treated with hypertonic solutions, plasma osmolality significantly increased time-dependently after injection as shown in Fig. 6. In the NaCl -treated group, the plasma osmolality increased rapidly within 20 min after injection and then slowly rose to approximately 420 mOsmol/L. In the other two groups treated with Na_2SO_4 and glucose respectively, the plasma osmolality was successively elevated to approximately 410 and 430 mOsmol/L, respectively.

Thus, the value of plasma osmolality at death did not significantly differ among these three groups.

Fig. 7 shows the time-dependent changes in plasma sodium concentration following intraperitoneal injection of the hypertonic solution. The sodium concentration showed a significant change in plasma even 5 min after injection of the hypertonic solution in all three drugs, although in a different way for each. The sodium level was linearly elevated to approximately 250 mEq/L in the Na_2SO_4 group, while in the NaCl group, the sodium level increased slowly to 200 mEq/L and formed a plateau at 30 min after injection. In the glucose group, however, the sodium level decreased significantly at 5 min

after injection, showing a gradual decrease to approximately 120 mEq/L.

The chloride concentration also increased time-dependently in parallel with that of sodium in the NaCl group, but decreased in the Na_2SO_4 and glucose groups as shown in Fig. 8.

Fig. 9 indicates the time-dependent changes in the plasma levels of potassium in the respective groups of rats treated with the three types of hypertonic solutions. In the Na_2SO_4 group, the potassium level was elevated linearly to approximately 10 mEq/L within 40 min after injection. The glucose-treated rats showed the same pattern of increase as that of the Na_2SO_4 -treated rats in the plasma level of potassium to 10 mEq/L. The plasma level of potassium in the NaCl group also elevated more slowly to reach

Fig. 7. Time-dependent changes in the sodium concentration of plasma in rats injected intraperitoneally with various hypertonic solutions. See explanation in Fig. 6.

Fig. 8. Time-dependent changes in the chloride concentration of plasma in rats injected intraperitoneally with various hypertonic solutions. ** and ***; $p < 0.01$ and $p < 0.001$. See explanation in Fig. 6.

Increased plasma potassium by hypertonic solution

a maximal value of 10 mEq/L. It was characteristic of the results that the plasma level of potassium increased to 10 mEq/L at the time of death in all rats subjected to an intraperitoneal injection of hypertonic solution irrespective of the drug used.

Furthermore, as seen in Fig. 9, the more rapidly the potassium concentration in rat plasma increased, the earlier the rats died.

Fig. 9. Time-dependent changes in the potassium concentration of plasma in rats injected intraperitoneally with various hypertonic solutions. *and***: Significant differences from the value at 0 min ($p < 0.05$ and $p < 0.001$, respectively).

see explanation in Fig. 6.

DISCUSSION

From the results obtained in our experiments, it is evident that a hypertonic solution injected intraperitoneally induced rapid exchange in water and solutes across peritoneum, thus causing an increase in abdominal fluid volume and plasma osmolality. Although the osmolar concentrations of the three kinds of solutions (glucose, NaCl and Na₂SO₄) were all 2800 mOsmol/L, the glucose solution caused the most intensive increase in ascites as shown in Fig. 1. This indicated that the attraction of water toward abdominal cavity occurred more rapidly and far more intensively in case of glucose than in cases of NaCl and Na₂SO₄. Notwithstanding, the average death time of the glucose group was rather prolonged as compared with those of the other two groups in Exam 1.

Accordingly, it is not suggested that the attraction of water from blood was the only direct cause for death.

Finberg et al. (1959) and Luttrell et al. (1959) pointed out that experimentally induced hypernatremia caused hemorrhagic encephalopathy and death. Takeuchi et al. (1980) also demonstrated that hypertonic solutions of glucose, NaCl and Na₂SO₄ each caused death accompanied by intracranial bleeding. As shown in Fig. 7, glucose did not produce natremia but did cause hyponatremia when given in a high concentration; the

administration of NaCl produced a continuous hypernatremia in the rats in Exam 2, which, however, survived longer than the glucose group in Exam 2. The chloride pattern in plasma moved in accord with sodium. Consequently, it can be seen that hypernatremia as well as hyperchloremia is not an essential factor in causing intracranial bleeding and death.

The most interesting observation was that a marked potassemia was produced by intraperitoneal injections of hypertonic solutions. As shown in Fig. 8, the potassium concentration in plasma increased rapidly and went up to approximately 10 mEq/L in all of cases given hypertonic solutions of glucose, NaCl and Na₂SO₄, respectively. For instance, the rats given NaCl in Exam 2, showed a retardation of the increase in plasma potassium and accordingly their death was prolonged until the potassium level reached the maximal value of 10 mEq/L.

It can be seen, therefore, that elevation of the potassium level in plasma is closely related to the cause of death and that the critical level of potassium for death in this case was 10 mEq/L.

The increased potassium in ascites is suggested to result from the plasma potassium which moves into ascites to replace solutes transferred to plasma.

Sotos et al. (1960, 1962) claimed that cell damage induced by hypertonic body fluid resulted in an efflux of hydrogen ions as well as potassium, phosphorus and organic acid into extracellular fluid. If this is so, the increased potassium in plasma may originate from damaged cells which have possibly been induced by hypertonic body fluid. As a matter of fact, the osmolality of plasma was greatly increased in rats which were subjected to the administration of hypertonic solution as seen in Fig. 6.

However, the fact that there exists no parallel relation between time-dependent changes in plasma osmolality and in potassium level, is worth notice. While the increase in osmotic pressure of plasma occurred quickly and reached a maximum at 20 min after administration of NaCl solution as seen in Fig. 6, the level of potassium elevated slowly to reach its maximum at 90 min after administration as shown in Fig. 8. This information suggests a dissociation of experimental results between plasma osmolality and potassium level.

It seems likely, therefore, that there are some other unknown factors involved in the induction of hyperpotassemia by hypertonic solutions.

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