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Diabetogenic Effect and Inhibition of Insulin Secretion Induced in Normal Rats by Ammonium Infusions*

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Summary. In order to explain the abnormalities of glucose metabolism previously observed in patients with blood ammonia elevation, the effect of a transitory hyperammonemia on I. V. glucose tolerance was investigated in rats. An I. V. glucose tolerance test was performed in 3 groups of 15 rats 60 min after the beginning of a 95 min infusion of either a 2 ml isotonic NaCl solution (control group) or ammonium acetate solutions at low (0.50 μmol/kg/min. NH_4+) or high doses (1.70 μmol/kg/min NH_4+). The "high" NH_4+ infusion produced an increase of blood ammonia to levels near 1000 μg/100 ml, a significant decrease in the K coefficient for glucose disappearance ($2.53 \times 10^{-2} \pm 0.20$ compared to $4.92 \times 10^{-2} \pm 0.13$ in control group) and a suppression of the radioimmunological plasma insulin (I. R. I.) response to glucose. With the "low" NH_4+ infusion the hyperammonemia was less pronounced (200–300 μg/100 ml), but the decrease in K($3.02 \times 10^{-2} \pm 0.15$) and in the first phase of I. R. I. release remained significant. The decrease in glucose disappearance rate could be accounted for by the proportional decrease in insulin secretion. Thus glucose intolerance induced by ammonium acetate infusions may be due to a direct effect of NH_4+ on the pancreas. These abnormalities in glucose metabolism depend on the quantity of infused ammonium.

Key words: Rat, ammonium infusion, blood ammonia, glucose metabolism, plasma immunoreactive insulin.

In 1955 Bessman *et al* [4] suggested that the neurotoxicity of hyperammonemia in porto-systemic encephalopathy results from a disturbance in cerebral glucose utilization. The observation, in hepatic coma, of a decrease in cerebral oxygen consumption [12, 22] and an increase in some intermediates of glucose metabolism [11, 35] supports this hypothesis. However the influence of variations in ammonium metabolism on the regulation of carbohydrate metabolism is still debated [10, 34]. Recently, we noted that I. V. glucose tolerance was worse in cirrhotic patients with hepatic coma than in those without porto-systemic encephalopathy [30]. One factor which could explain this observation, was the increase in arterial blood ammonia which was consistently observed during hepatic coma. Thus we noted in both normal and cirrhotic subjects, infused with ammonium chloride or acetate, that a transitory elevation in arterial blood ammonia invariably brought about a very significant diminution in I. V. glucose tolerance [31] and an inhibition of the insulin secretion induced by the glucose load [32]. However, in these studies, we were unable to show any correlation between these anomalies and the blood ammonia levels. The aim of the present study was to compare in normal rats the action of a moderate or gross hyperammonemia on glucose tolerance and insulin secretion.

Material and Methods

Three groups of 15 male Wistar rats, weighing 250–300 g and fasted for 20 hours, were used. After brief anesthesia with ether, a polyethylene catheter was introduced into the jugular vein and the anesthesia was continued with sodium pentobarbital, according to need (3–5 mg/100 g – body weight in 95 min). Then, a tracheotomy was performed and a carotid artery was catheterized to take blood samples and to measure regularly the blood pressure using a Statham manometer. Finally, a catheter placed in the femoral vein was used to inject the test solution.

In the first (control) group of 15 rats, 2 ml of a 0.9 g/100 ml NaCl solution were infused for 95 min, using an infusion pump. In the second group, this NaCl solution was replaced by an isotonic ammonium acetate solution, which was perfused at a low dose of 0.50 μmol/kg body weight/min of ammonium (NH_4+). The third group was infused with an ammonium acetate solution at a high dose of 1.70 μmol/kg/min NH_4+. The pH, at 37° C, of these two

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solutions was 6.7. A sodium acetate solution was not used in the control group because its pH (7.82) was considered to be too high.

The blood glucose and the radio-immunologic plasma insulin (I. R. I.) were determined before and at the 60th min of the infusion. The blood ammonia was measured every 10 min during this period. At the 60th min of the infusion, an I. V. glucose tolerance test was performed in all the rats by the injection, over 30 sec, of 1 g glucose/kg body weight, using a 50 g/100 ml solution [7]. After the beginning of this injection, the blood sugar was measured every 5 min for 35 min, the I. R. I. at the 4th, 10th, 20th and 35th min and the blood ammonia at the 5th, 10th and 35th min. Five rats infused with the "high" NH_4+ solution had their arterial pH measured beforehand, at the 60th min and at the end of the infusion, using 0.1 ml of blood and a type 27 Radiometer apparatus. The blood glucose levels were measured on 0.05 ml of blood by the technique of Hoffman [18] adapted to a Technicon autoanalyzer. The ammonia was also determined on 0.05 ml of blood using a method of automatic dialysis [20]. For both of these determinations, the blood was pumped directly from the carotid of the rat to the autoanalyzer to avoid any intermediate manipulation and any excessive blood loss. The insulin levels were determined on 0.1 ml of plasma by the radio-immunological method of Hales and Randle [15] – the standard curves being established from rat insulin. The sensitivity of the method was 5 to 200 μU/ml. All the measurements were made with the same kit and the relative precision was about 10% [28]. The results of the blood sugar and I. R. I. levels allowed us to calculate in each case, by computer programme, the K coefficient for glucose disappearance from the 5–30 min. glucose values, using the formula of Amatuzio [3], and the areas of the plasma insulin and blood sugar levels whose ratio corresponds to the insulinogenic index [33].

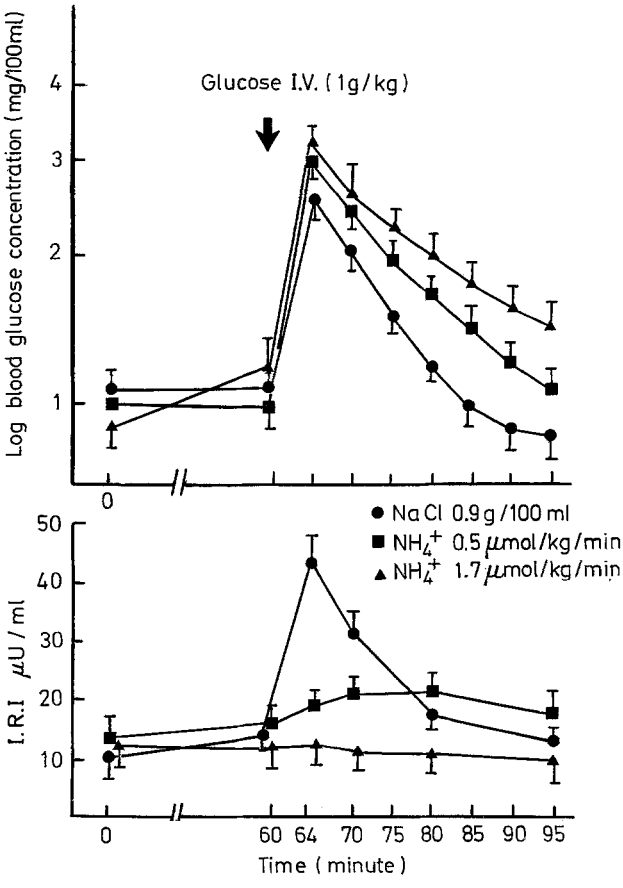

Fig. 1. Blood glucose and plasma I. R. I. changes before and after an I. V. glucose load in rats infused with either isotonic sodium chloride or ammonium acetate solutions. Each point represents the mean ± SEM of 15 experiments

During the experiment the total blood loss was 2.2 ml and was replaced by the injection of the equivalent volume of a 0.9 mg/100 ml NaCl solution containing heparin (25 mg/100 ml). This avoided any coagulation during blood sampling and any significant change in blood pressure.

All the results are expressed in mean values ± S.E.M. and were analyzed using techniques of variance analysis, linear regression and Student's "t" test.

Results

1. Arterial Blood Ammonia

In the control group, the basal arterial ammonia was 51 ± 2.50 μg/100 ml with no change during the NaCl infusion. During the "low" NH_4+ infusion, the level increased rapidly to 250 ± 12 μg/100 ml at 60 min and to 275 ± 15 at 95 min. These arterial levels were comparable to those usually observed in cirrhotic patients in hepatic coma [20]. This hyperammonemia was not modified by the rapid injection of glucose at the 60th min.

The "high" NH_4+ infusion brought about a more marked hyperammonemia, which reached 926 ± 60 μg/100 ml at 60 min and 1010 ± 68 at 95 min. It was accompanied by a temporary hyperpnea, a moderate but non significant decrease in the mean blood pressure and a decrease in the sodium pentobarbital doses necessary to maintain the anesthesia (3 mg/100 g of body weight in contrast to 5 mg/100 g in the two other groups of animals). These modifications appeared to be related to the degree of ammonia intoxication. We also noted a slight elevation in arterial pH (7.44 ± 0.01 before the infusion; 7.47 ± 0.03 at the 60th min; 7.48 ± 0.02 at the end). This last modification was seemingly linked to the hyperpnea which may have resulted from stimulation of the respiratory centre by the ammonium infusion [27].

2. Blood Glucose and K Coefficient of Glucose Disappearance

The NaCl and "low" NH_4+ infusions brought about no variation in the blood glucose after 60 min. But the blood glucose levels did increase significantly during the "high" NH_4+ infusion, rising from 98 ± 3 to 127 ± 7 mg/100 ml at the 60th min. ($p<0.01$) (Fig. 1).

After the rapid I. V. glucose load, all the blood sugar levels were higher in rats perfused with NH_4+ than in the control animals (Fig. 1). However, statistically significant differences ($p<0.01$) were only found between the blood glucose mean values of the control group and those of the group infused with the "high" dose of NH_4+ at the 15th, 20th, 25th, 30th and 35th min after the glucose injection.

On the other hand, the K coefficient was significantly decreased after the "low" NH_4+ infusion ($K = 3.02 \times 10^{-2} \pm 0.15$; $p<0.001$) and the "high" NH_4+ infusion ($2.53 \times 10^{-2} \pm 0.2$; $p<0.001$) as compared to the K determined in the control animals ($K = 4.92 \times 10^{-2} \pm 0.13$). When the rate of infused ammonium increased, then the K coefficient decreased proportionately. In addition, the area of blood glucose (Fig. 2) was significantly higher in the two groups of rats infused with ammonium than in the control animals ($p<0.01$).

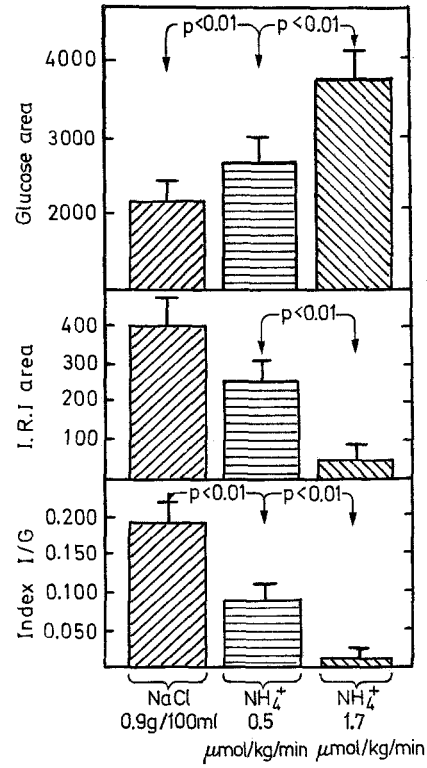

Fig. 2. Mean values (± SEM) of the blood glucose and plasma I.R.I. areas and of the insulinogenic index (I/G) determined in the 3 groups of rats after the I. V. glucose load (Areas are expressed in arbitrary values)

3. Insulin Levels

(Fig. 1). The I. R. I. mean values 60 min after the start of the perfusion (11.7 μU/ml ± 1.5) were not significantly different from the initial levels (12.5 μU/ml ± 1) in any of the three groups.

In the control animals, the I. R. I. rose to 42 ± 3.8 μU/ml 4 min after the glucose injection and fell to 30 μU/ml ± 4.0 at the 10th min. The I. R. I. values at 4, 10 and 20 min were higher than the basal level ($p<0.01$).

During the low NH_4+ infusion, the I. R. I. rose only to 17.3 ± 2.9 μU/ml at the 4th min., but later continued to increase to 18.5 ± 1.9 at the 10th min and to 20.5 ± 2.6 at the 20th min. All these levels were higher than the basal I. R. I. ($p<0.01$). Likewise, corresponding to the decrease in the first phase of the insulin release, there was a significant reduction in the plasma insulin area. This contrasted with the increase of the blood glucose area which resulted in a marked reduction of the insulinogenic index ($p<0.001$) (Fig. 2).

On the other hand, in the rats infused with the "high" NH_4+ infusion, we did not observe any insulin response following the glucose load and the insulinogenic index tended toward 0. It appears then that the insulin secretion was lowered in proportion to the quantity of infused ammonium ($p<0.01$). Moreover, for the 3 groups of rats, there existed a negative linear correlation between the I. R. I. values at 4 min and the logarithm of the arterial blood ammonia levels at the same time ($r = 0.751$; $p<0.001$) (Fig. 3). The inhibiting effect of hyperammonemia on insulin secretion was directly related according to an exponential function. There was also a linear correlation ($r = 0.512$; $p<0.001$) between the I. R. I. values at 4 min and the K coefficient; consequently, the latter seemed to depend directly on the first phase of insulin release. Finally, a linear regression between the I. R. I. and blood glucose levels at 4, 10 and 20 min was only found in the control group ($r = 0.653$; $p<0.001$); in the two other groups the ammonium infusions significantly modified the insulin regulation of carbohydrate metabolism.

Discussion

These results lead us to conclude that an infusion of ammonium acetate in rats brings about an alteration in carbohydrate metabolism and its regulation. The results are, on the whole, similar to those we have recently observed in normal or cirrhotic humans after

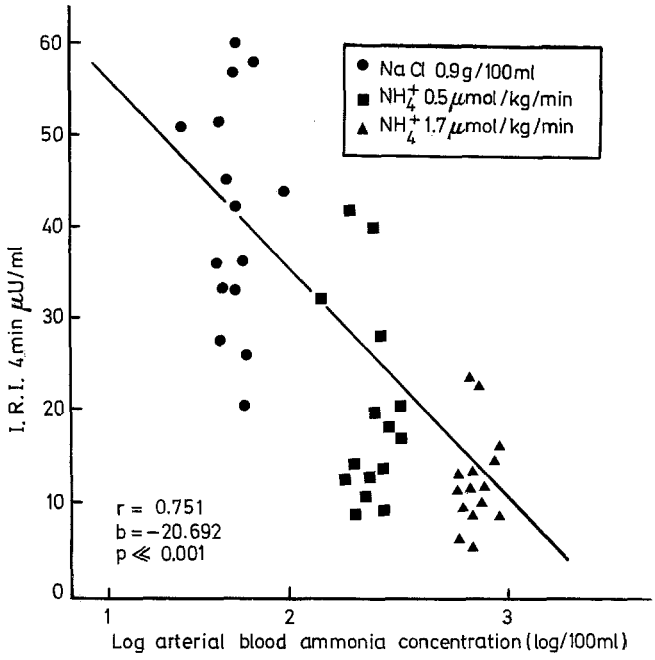

Fig. 3. Relationship between the plasma I. R. I. values at the 4th min after the I. V. glucose load and the logarithm of the arterial blood ammonia concentration at the same time

the infusion of either ammonium chloride or ammonium acetate [31]. Thus it is unlikely that either the acetate radical or the chloride ion have any influence on the genesis of these anomalies, thereby implicating the ammonium ion. In the present study, we have used only ammonium acetate solutions in order to avoid the acidifying action of ammonium chloride, because it has been suggested that metabolic acidosis could have a diabetogenic action [2, 21]. In order to exclude an effect of the acetate radical, we have found, in additional experiments, that in 5 normal rats perfused with a sodium acetate solution (8 μmol/kg/min) the K coefficient of glucose disappearance ($4.58 \times 10^{-2} \pm 0.16$) was similar to that observed in the control group infused with the NaCl solution.

Hyperammonemia may affect glucose metabolism by combination with an intermediate of the Krebs cycle such as alpha-ketoglutaric acid [4] or by stimulation of glycolysis, as seen in the brain [17, 23, 29]. It is also possible, according to the hypothesis of Brown *et al.* [6], that urea cycle stimulation by hyperammonemia could inhibit the tricarboxylic cycle. Hyperammonemia may also stimulate the Na-K dependent ATPase and therefore alter the cell membrane transport of glucose [29].

Clifford *et al.* [8, 25] have also observed an increase in glycogenolysis with hyperglycemia in rats rendered hyperammonemic by sublethal urease injections. After removal of the adrenal glands, however, the increase in glycogenolysis was smaller. This finding leads us to believe that the diabetogenic potential of ammonia may result, in part at least, by the intermediary of the catecholamines. However, in the ammonia intoxications caused by urease injections, one cannot eliminate with certainty any particular influence of urease or other metabolite arising from its action [5].

On the other hand, our results show that ammonium infusions have an inhibiting effect on the insulin secretion induced by a glucose load. We have already made similar observations in man [32], but the present study demonstrates that in the rat the inhibition of insulin secretion is in proportion to the degree of induced hyperammonemia: in small quantity, ammonium slows down the first phase of insulin release; in larger quantities, it suppresses all insulin secretion, except perhaps before the 4th min where I. R. I. was not measured. Ammonium effect appears then to be both modulating and inhibiting.

The inhibiting effect of ammonium on insulin secretion was also shown by Feldman and Lebowitz [13], who noted, on isolated and perfused hamster pancreas, that ammonium ions inhibited only the insulin secretion induced by a glucose stimulus, but not that following a Tolbutamide injection. This suggests that ammonium acts either by interfering with the action of glucose on the islets, or by modifying the ionic membrane exchanges which are very important in the regulation of insulin secretion [14, 16, 19]. Cellular potassium depletion secondary to an intra-cellular ammonium accumulation [1] supports the latter hypothesis because it suggests an action of ammonium intoxication on the sodium pump. However, addition of phentolamine to the preparation of Feldman and Lebowitz [13] suppressed the inhibitory effect of NH_4 + on insulin secretion. This fact, as well as Prior's *in vivo* observations [25] after removal of the adrenal glands, suggests that hyperammonemia stimulates catecholamine secretion. This would provoke hyperglycaemia and inhibit insulin secretion [24].

The inhibitory effect of ammonium on insulin secretion must be partly responsible for the glucose intolerance. In fact, for all the rats, we found a significant correlation between the values of the K coefficient and the I. R. I. at 4 min and it is well established that after an I. V. glucose load the blood glucose variations depend on both the tissue uptake of insulin and on the first phase of insulin release [9].

However, the diabetogenic action of ammonium does not seem to result only from the inhibition of insulin secretion. Thus, the hyperglycaemia, without any modification in the I. R. I. levels, that we observed at the 60th min of the "high" NH_4+ infusion and the reduction in the response to exogenous insulin in normal or alloxan-diabetic rats with hyperammonemia noted by Prior [25] suggest a direct action of

ammonium on carbohydrate metabolism. Hyperammonemia might also exert a diabetogenic action via peripheral insulin resistance.

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