# Effects of infusions of lysine, leucine and ammonium chloride into the hepatic portal vein of chickens on voluntary food intake

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1. Adolescent cockerels of a laying strain were prepared with catheters whose tip lay in the hepatic portal vein, to study the effect of 3-h infusions of nutrients on food intake.

2. Lysine, infused into the hepatic portal vein at rates of 150–450 mg/3 h reduced 3-h food intake by up to 58%, for a period of 6 h in previously starved birds, but had no effect on birds allowed free access to food. Infusions made into the jugular vein had no effect, suggesting a role for the liver in monitoring lysine levels.

3. Portal infusion of leucine had a delayed effect while ammonium chloride, infused at isomolar rates to those of the lysine infusions, had very little effect on intake.

4. The results support the concept of liver sensitivity to amino acids, but the mode of action is not clear; it appears not to be via the effects of ammonia.

There is considerable evidence that absorbed metabolites are important in the control of food intake in mammals, with glucose having been implicated for several decades (Mayer *et al.* 1951). Almquist (1954) suggested that there was a correlation between plasma amino acid concentrations and food intake; infusion of a single or imbalanced amino acid mixture, or feeding a high-protein or imbalanced diet raises plasma amino acid concentrations and leads to a decrease in food intake (Mellinkoff *et al.* 1955; Sanahuia *et al.* 1965; Peng & Harper, 1969; Harper *et al.* 1970; Ashley & Anderson, 1977). Panksepp & Booth (1971) injected a balanced amino acid solution into the hypothalamus and depressed food intake, suggesting a role for the brain in monitoring raised amino acid levels.

Russek (1970), however, demonstrated that infusion of glucose or ammonium chloride into the hepatic portal vein reduced food intake; the infusion had no effect when made into the jugular vein, suggesting that the liver monitored glucose and ammonia levels. Yin *et al.* (1979) also showed that a balanced amino acid solution (50 g/l), infused into the mesenteric vein of the rat, reduced food intake.

The previous work has been carried out on mammals. There has been less work with chickens, infusion of glucose into peripheral veins having little effect (Smith & Bright-Taylor, 1974). Glucose infused into the portal vein in physiological concentrations does, however, depress food intake as does a balanced mixture of amino acids (Shurlock & Forbes, 1981, 1984), while similar infusions into the jugular vein have little or no effect. However, the infusion of a balanced mixture of amino acids into the liver does not indicate whether food intake is being reduced by the stimulation of specific amino acid receptors, or whether glucose receptors are being stimulated by glucose synthesized from gluconeogenic amino acids. The amino acid required in greatest quantities by the chicken is lysine (Agricultural Research Council, 1975) and this has been shown to depress intake when infused at 100 mg/h into the carotid artery or jugular vein (Tobin & Boorman, 1979). In the present study, the first three experiments investigated its effects on food intake during and after infusion into the hepatic portal vein. Lysine is a gluconeogenic amino acid and its infusion could stimulate both types of receptors, whereas leucine is different both chemically and metabolically; it donates its amino group to pyruvate-producing alanine in mammals. This takes place largely in muscle, some of the alanine then being transported

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in blood to be metabolized by the liver. The effect of leucine is compared with that of lysine in the fourth experiment. If amino acid receptors do exist, then, rather than having twenty different receptors to measure each amino acid, it would seem more likely that a common chemical group is being monitored. In birds, which do not have a urea cycle, the most likely group is $NH_3$ and $NH_4Cl$ was infused into the hepatic portal vein in the fifth experiment.

## EXPERIMENTAL

Cockerels of an egg-laying strain (404; Mytholmroyd Hatcheries Ltd, Hebden Bridge, W. Yorks.) were housed individually in a windowless room at a constant 19° and 17 h artificial light per 24 h, the photoperiod starting at 06.15 hours. Each bird was prepared under general anaesthesia (equithesin; Gandal, 1969) with a catheter with one end positioned in the hepatic portal vein and the other exteriorized at the top of the head (Rusby 1982). The birds were allowed free access to food (Poultry Groaster Pellets; E. B. Bradshaw and Sons Ltd, Bell Mills, Driffield) and intake was measured at regular intervals.

Infusions were made through polyethylene tubing attached to a cannula on top of the bird's head, and leaving the cage through a hole in the top where the pump was situated. Infusions started at 11.00 hours and continued for 3 h.

In each experiment the treatments were given in a randomized row and column design in which each bird was subjected to a different treatment in any 1 d. The results were subjected to generalized linear model analysis to test for significant differences between treatments (S.A.S. 1982) with between-bird and between-day effects accounted for. This allowed for unequal numbers of observations in cases where some birds did not complete the full range of treatments. Standard errors were calculated from the residual mean square and were used to calculate least significant differences.

### *Expt 1. Infusion of lysine into the hepatic portal vein*

Eight cockerels, aged 16 weeks and weighing 2·0–2·7 kg, were prepared with hepatic portal vein catheters. They received the following treatments in turn, infused at a rate of 1·0 ml/h between 11.00–14.00 hours, the catheters being primed with the solution to be infused: (1) 0·1 M-phosphate buffer (pH 7·4, osmotic pressure 310 mosmol/l), i.e. 0 mg lysine/3 h; (2) 0·8 mmol lysine in 0·1 M-phosphate buffer (pH 7·4, osmotic pressure 312 mosmol/l), i.e. 150 mg lysine/3 h; (3) 1·6 mmol lysine in 0·1 M-phosphate buffer (pH 7·4, osmotic pressure 312·5 mosmol/l), i.e. 300 mg lysine/3 h; (4) 2·4 mmol lysine in 0·1 M-phosphate buffer (pH 7·4, osmotic pressure 312·9 mosmol/l), i.e. 450 mg lysine/3 h.

The birds were allowed at least 48 h between each treatment. Earlier experiments had shown that this gave the birds sufficient time to recover from the previous treatment. The experiment was repeated with the same birds but in some cases the portal catheters blocked before the end of the experiment, with the result that eleven, nine, twelve and twelve observations were made for the four treatments respectively.

### *Expt 2. Infusion of lysine into the hepatic portal vein of 21-h starved birds*

Previous work (Rusby, 1985) had shown a much greater depression of food intake by portal infusion of metabolites in birds which had been fasted overnight. This experiment was therefore similar to Expt 1, but with food withheld for 21 h before each infusion and with at least 72 h between each treatment to allow recovery after the previous treatment and with each bird used only once. Six cockerels, 21 weeks old and weighing 2·8–3·1 kg with hepatic portal vein catheters, were used. One catheter blocked before the end of the experiment, resulting in five observations for the 300 mg lysine dose.

*Expt 3. Lysine infusion into the hepatic portal vein or the jugular vein on the food intake of 21-h starved birds*

Six cockerels, aged 16 weeks and weighing 1·8–2·3 kg, were prepared with hepatic portal vein and jugular vein catheters. The following treatments were given: (1) 0·1 M-phosphate buffer (pH 7·4, osmotic pressure 310 mosmol/l) into both hepatic portal vein and jugular vein; (2) 1·6 mmol lysine in phosphate buffer (pH 7·4, osmotic pressure 312·5 mosmol/l) into the hepatic portal vein and phosphate buffer into the jugular vein; (3) phosphate buffer (0·1 M, pH 7·4) into the hepatic portal vein and 1·6 mmol lysine in phosphate buffer into the jugular vein.

The experiment was repeated with the same birds but again two catheters blocked before the end of the experiment, resulting in ten, eleven and ten observations for the three treatments respectively.

*Expt 4. The infusion of leucine and lysine into the hepatic portal vein of 21-h starved birds*

Eight cockerels, weighing 2·7–2·9 kg and aged 18 weeks, were prepared with hepatic portal vein catheters. The experiment was carried out as in Expt 1 but the infusion rate was increased to 10 ml/h because of the low solubility of leucine. Preliminary infusions of phosphate buffer at this rate showed that it affected food intake, so the amino acids were dissolved in isotonic saline (9 g sodium chloride/l). The solutions infused were as follows: (1) isotonic saline; (2) 1·6 mmol leucine (210 mg leucine/h) (isomolar with the lysine infusion); (3) 3·2 mmol leucine (420 mg leucine/3 h) (isonitrogenous with the lysine infusion); (4) 1·6 mmol lysine (300 mg lysine/3 h).

The experiment was repeated with the same birds but two catheters blocked before the end of the experiment, giving sixteen, fifteen, fourteen and fifteen observations for each treatment respectively.

*Expt 5. The infusion of $NH_4Cl$ into the hepatic portal vein of 21-h starved birds*

Seven cockerels, weighing 2·0–2·4 kg and aged 15 weeks, were prepared with hepatic portal vein catheters and allocated to two experimental blocks. The solutions infused were as follows: (1) isotonic saline; (2) 1·6 mmol $NH_4Cl$ (96 mg/3 h); (3) 3·2 mmol $NH_4Cl$ (192 mg/3 h); (4) 4·8 mmol $NH_4Cl$ (288 mg/3 h).

The experiment was repeated with the same birds but not all the birds reached the end of the experiment because of blocked catheters, resulting in thirteen, thirteen, eleven and eleven observations for each treatment respectively.

## RESULTS

### *Expt 1*

There was no significant effect of lysine infusion on the food intake of birds fed *ad lib*; mean intakes during the 0, 150, 300 and 450 mg lysine/3 h treatments were respectively: 22·3, 22·8, 19·5 and 24·2 g/3 h (SEM 2·33 g/3 h).

### *Expt 2*

Lysine infusion reduced food intake in a dose-related manner in 21-h starved birds (Table 1).

It was in the 2nd and 3rd hours of infusion, when the initial desire to eat after a period of starvation had been overcome, that the greatest effect was seen. This effect persisted during the 3 h after infusion and to a lesser extent after 24 h.

Table 1. *Expt 2. Food intake (g) of six 21-h fasted birds with infusion of four levels of lysine into the hepatic portal vein*

| | Rate of infusion (mg lysine/3 h) | | | | |
|---|---|---|---|---|---|
| No. of observations ... | 0<br>6 | 150<br>6 | 300<br>5 | 450<br>6 | SEM |
| Time-period after onset of infusion (h): | | | | | |
| 0–1 | 48·5[a] | 50·7[a] | 28·0[a] | 33·2[a] | 6·95 |
| 1–2 | 16·3[a] | 4·8[b] | 5·8[b] | 1·5[b] | 2·74 |
| 2–3 | 8·5[a] | 3·5[b] | 1·8[b] | 0·7[b] | 1·47 |
| 0–3 | 73·3[a] | 59·0[ab] | 35·6[b] | 33·5[c] | 4·73 |
| 3–6 | 16·2[a] | 8·8[b] | 7·0[b] | 1·3[c] | 1·66 |
| 0–24 | 136·3[a] | 114·2[a] | 86·4[a] | 96·7[a] | 13·33 |

[a, b, c] Within each time-period, means with the same superscript letter did not differ significantly ($P > 0·05$).

Table 2. *Expt 3. Food intake (g) of six 21-h fasted birds with infusion of lysine into the hepatic portal vein (HPV) or into the jugular vein (JV)*

| | Rate of infusion (mg lysine/3 h) | | | |
|---|---|---|---|---|
| No. of observations ... | 0 HPV<br>0 JV<br>10 | 300 HPV<br>0 JV<br>11 | 0 HPV<br>300 JV<br>10 | SEM |
| Time-period after onset of infusion (h): | | | | |
| 0–1 | 33·7[a] | 24·7[a] | 31·8[a] | 4·58 |
| 1–2 | 8·1[a] | 5·7[a] | 5·9[a] | 1·46 |
| 2–3 | 5·8[a] | 3·1[a] | 5·2[a] | 1·30 |
| 0–3 | 47·6[a] | 33·5[a] | 42·9[a] | 5·26 |
| 3–6 | 17·8[a] | 5·8[b] | 10·7[ab] | 3·09 |
| 0–24 | 99·1[a] | 77·4[a] | 98·3[a] | 7·27 |

[a, b] Within each time period means with the same superscript letter did not differ significantly ($P > 0·05$).

Table 3. *Expt 4. Food intake (g) of eight 21-h fasted birds receiving leucine or lysine into the hepatic portal vein*

| | Rate of infusion (mg/3 h) | | | | |
|---|---|---|---|---|---|
| No. of observations ... | 0<br>16 | 210<br>Leucine<br>15 | 420<br>Leucine<br>14 | 300<br>Lysine<br>15 | SEM |
| Time-period after onset of infusion (h): | | | | | |
| 0–1 | 28·4[ab] | 25·6[b] | 38·0[a] | 18·0[b] | 1·95 |
| 1–2 | 12·4[a] | 13·7[a] | 5·0[b] | 2·2[b] | 2·10 |
| 2–3 | 8·8[ab] | 14·3[a] | 9·6[ab] | 2·4[b] | 2·22 |
| 0–3 | 49·6[a] | 53·6[a] | 52·6[a] | 22·6[b] | 2·49 |
| 3–6 | 20·2[a] | 17·7[a] | 3·7[b] | 16·2[a] | 4·62 |
| 0–24 | 108·2[a] | 127·0[a] | 100·0[a] | 99·40[a] | 12·78 |

[a, b] Within each time-period, means with the same superscript letter did not differ significantly ($P > 0·05$).

Table 4. *Expt 5. Food intake of seven 21-h fasted birds receiving four levels of ammonium chloride infused into the hepatic portal vein*

| | Rate of infusion (mg $NH_4Cl$/3 h) | | | | |
|---|---|---|---|---|---|
| | 0 | 96 | 192 | 288 | SEM |
| No. of observations ... | 13 | 13 | 11 | 11 | |
| Time-period after onset of infusion (h): | | | | | |
| 0–1 | 37·0 | 38·0 | 30·7 | 33·6 | 2·98 |
| 1–2 | 8·8 | 5·5 | 7·1 | 7·0 | 1·54 |
| 2–3 | 6·1 | 9·3 | 7·7 | 3·3 | 1·59 |
| 0–3 | 51·8 | 52·9 | 45·5 | 43·9 | 3·77 |
| 3–6 | 25·8 | 24·5 | 27·0 | 18·5 | 3·08 |
| 0–24 | 153·6 | 158·5 | 154·5 | 135·2 | 7·22 |

None of the values differed significantly ($P > 0·05$).

*Expt 3*

Infusion of lysine into the portal vein tended to cause a greater reduction in food intake than when the infusion was made into the jugular vein, but neither of the effects was significant during the infusion (Table 2). However, intake was significantly depressed by lysine given into the hepatic portal vein during the 3 h after the infusion, and almost significantly over the 24 h after the start of infusion ($P = 0·065$).

*Expt 4*

There was no significant effect of leucine on intake in the 1st hour of the infusion while lysine depressed intake during this period. During the 2nd hour both lysine and leucine at the higher dose depressed intake but only the effect of lysine persisted into the 3rd hour. There was no carry-over effect of lysine, while the higher dose of leucine significantly depressed intake during the 3 h after the end of the infusion. Total daily intake was not affected by treatment (Table 3).

*Expt 5*

Infusion of $NH_4Cl$ into the portal vein had no significant effect on food intake over the range of doses used (96–288 mg/3 h) (Table 4).

## DISCUSSION

Infusion of lysine into the hepatic portal vein reduced the weight of food eaten by cockerels, but only after a 21 h fast and not when free access to food was allowed before infusion. The requirement for lysine is approximately 60 mg/kg per 24 h (Agricultural Research Council 1975), so these cockerels weighing 2·0–2·7 kg required 120–162 mg/24 h. During the infusion they received 150–450 mg lysine, a level which when added to that being absorbed from the food eaten, would give considerably elevated lysine levels in the blood. The large amounts of lysine required to depress intake significantly in fasted birds, and the lack of effect in free-fed birds, makes it difficult to assign a physiological role to lysine in the normal control of voluntary intake. However, Vanderweele *et al.* (1976) proposed that liver glycogen levels are important when considering the ability of the liver to monitor glucose levels, because in the fed state liver glycogen is high and glucose infusions have no effect on the food intake by rabbits, but in starved conditions liver glycogen is depleted and glucose infusions reduce food intake. Lysine is a gluconeogenic amino acid

which will partially contribute to the glucose pool, especially during and after a fast, and so may exert its effect via a 'glucostatic' mechanism working only in conditions following starvation. An alternative explanation is that in starved birds, where lysine-denaturing enzyme activity is reduced (Munro, 1965), infused lysine will not be degraded as quickly and so will have longer to exert its effect via a possible 'aminostat mechanism'.

The results of Expt 3 suggest that the liver may be the site of action of lysine, especially when considered along with evidence that vagotomy at the level of the proventriculus, which includes vagal branches to the liver, blocks the effects of portal vein infusion of lysine and glucose on intake (Rusby, 1985). This does not, however, rule out the likelihood of responses by other organs to lysine, as Tobin & Boorman (1979) have shown a greater depression of intake by cockerels when lysine was infused into the carotid artery than when given into the jugular vein at 100 mg/h, i.e. the same rate as treatment 3 in Expts 1 and 2.

It is possible that infusion directly into the liver stimulates the production of some metabolite which then exerts its affect elsewhere, possibly on the brain, this metabolite not being produced when the infusion is made into the jugular vein. This possibility has not been investigated further.

The results of Expts 1, 2 and 3 give no indication of the mechanism by which the lysine is exerting its effect, i.e. whether glucose or amino acid receptors exist. Expt 4 was designed to try and discover whether amino acid receptors do exist. Leucine is not taken up by the liver, but converted to alanine in muscle. Alanine in blood will be taken up by the liver and may be metabolized there. The lower level of leucine was chosen to give the same molar concentration of leucine as that of lysine in the lysine infusion, while the higher concentration would give the same amount of ammonia on degradation. The leucine requirement of chickens is 40 mg/kg body-weight per 24 h (Agricultural Research Council, 1975), and they received 210–420 mg, a level which would give greatly elevated leucine levels in the portal vein. The results of Expt 4 show that during the 1st hour, when food intake is probably influenced more by the availability of food after a period of starvation, the lysine infusion again significantly reduced food intake compared with saline controls, whereas leucine had very little effect initially. In the 2nd hour after the onset of infusion and during the 3 h after the end of infusion the higher dose of leucine did have a significant effect on food intake; this delay suggests that it is not the concentration of leucine *per se* which is being monitored but the concentration of the alanine produced. Prior *et al.* (1971) have suggested that $NH_3$ stimulates glycogenolysis and it may be that the $NH_3$ liberated from the breakdown of leucine (via alanine) has increased the intracellular glucose concentration. In Expt 4 the effect of lysine was not prolonged beyond the end of the infusion as it was in Expts 2 and 3; we have no explanation for this difference.

If amino acids in general are influencing the liver then the most likely metabolite to be monitored is $NH_3$, especially in birds as they lack a urea cycle. To investigate this, $NH_4Cl$ was infused in Expt 5 at levels comparable with the levels of $NH_3$ likely to be released from the previous amino acid infusions. There was no effect on food intake, indicating that the effects of amino acids on the liver are not mediated by $NH_3$.

These results agree with those of Leung & Rogers (1974) and Stephens & Baldwin (1974) who found no effect of amino acid or ammonium infusion into the portal vein of rats and pigs respectively. Russek (1971), Rezek & Novin (1977) and Yin *et al.* (1979) have shown a reduction in intake after portal infusion of amino acids and $NH_3$. However, these authors were using even greater quantities of nutrients than those employed in the experiments reported here.

In conclusion it would seem that the liver of fasted birds monitors nutrient intake and the results suggest that the receptors measure glucose concentration or uptake rather than that of amino acids of $NH_3$, the major product of catabolism of amino acids.

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