Free Amino Acids of Testes

CONCENTRATIONS OF FREE AMINO ACIDS IN THE TESTES OF SEVERAL SPECIES AND THE PRECURSORS OF GLUTAMATE AND GLUTAMINE IN RAT TESTES *IN VIVO*

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Determination of the free amino acid and lactate content of testicular tissue in rat, guinea pig, rabbit, cat, gerbil, hamster, chicken and bullfrog indicates a substantial species variation. Insulin hypoglycaemia and ammonium acetate toxicity changes the concentration of several free amino acids of rat testes. ^{14}C radioactivity from labelled acetate and ethanol is rapidly incorporated into some of the free amino acids of rat testes *in vivo*, whereas incorporation from [^{14}C]glucose is relatively slow. These results have been compared with those obtained from similar studies with rat brain. In contrast to brain, there is no evidence for glutamate compartmentation in testes.

Free amino acids have long been recognized as essential for the survival and motility of spermatozoa (Mann, 1964, p. 96). They have been proposed to act as buffering agents, metal-chelating agents, protective substances and extracellular utilizable substrates for the sperm cell (Mann, 1964, p. 166; Tyler & Rothschild, 1951; Giese & Wells, 1952; Bhargava *et al.*, 1959). The observation that after castration, vasectomy or vasoligation, the free amino acid content of seminal plasma is decreased to negligible amounts (Mann, 1964, p. 165; Hopwood & Gassner, 1962), suggests that testicular tissue is the main source of these amino acids. To date, only the free amino acid content of rat testes (Du Ruisseau *et al.*, 1957) and of bull testes (Melampy *et al.*, 1955) have been determined.

Testes have some similarity to brain in that both tissues have relatively high concentrations of free amino acids and a need for an adequate concentration of blood glucose. Insulin hypoglycaemia is damaging to both tissues. Studies by various investigators have suggested dependency of testicular tissue on glucose utilization (Schuler, 1944; Serfaty & Boyer, 1956; Mancini *et al.*, 1960; Free, 1970). No comprehensive study of comparison of possible precursors *in vivo* of free amino acids in testicular tissue has been reported.

Here we report the free amino acid and lactate content of testicular tissue of the rat, guinea pig, rabbit, cat, gerbil, hamster, chicken and bullfrog. The effect of insulin hypoglycaemia and ammonium acetate toxicity on the free amino acid content of rat

testes has been determined. Glucose has been compared with acetate and ethanol as a precursor of some of the free amino acids of testes *in vivo* and these results have been compared with those obtained from similar studies with rat brain.

Materials and Methods

Animals

Albino rats (180–250 g body wt.) were obtained from the Holtzman Co. (Madison, Wis., U.S.A.), guinea pigs (Hartley strain, 730–845 g body wt.) and Syrian hamsters (120–130 g body wt.) from the Hilltop Laboratory Animals Co. (Chatsworth, Calif., U.S.A.) and bullfrogs (13–18 cm in length) from E. G. Steinhilber Co. (Oshkosh, Wis., U.S.A.). Cats, gerbils, rabbits and chickens (Plymouth Rock) were obtained locally.

Rats were injected intraperitoneally with 0.3–0.8 ml of an aqueous solution of the labelled metabolite. Pyrazole at 6.6 mmol/kg was given intraperitoneally to rats (Mushahwar & Koeppe, 1972), who received [$1\text{-}^{14}\text{C}$]ethanol 20 min later. Some rats were given insulin intraperitoneally (150 units/kg body wt.) 150 min before being killed. The rats which were treated intraperitoneally with lethal doses (10.8 mmol/kg body wt.) of ammonium acetate were near death when they were killed 7–8 min later.

All rats (except as indicated in Table 1), gerbils and hamsters were killed by freezing in liquid N_2 . After being killed by decapitation or a blow on the head, testes were surgically removed (in less than 2 min)

Table 1. Free amino acid and lactate concentration ($\mu\text{mol/g wet wt.}$) of decapsulated (epididymis removed) testes of various animalsFor details see the text. The numbers of animals used are given in parentheses. The values are given as means $\pm$ s.e.m. N.D., not determined.

Amino acid and lactate concentration ($\mu\text{mol/g wet wt.}$)											
Amino acid		Rat (6)	Gerbil (3)	Guinea pig (3)	Hamster (3)	Rabbit (2)	Cat (2)	Rooster (3)	Frog (1)		
Phosphorylethanolamine		1.2 $\pm$ 0.2	4.2 $\pm$ 0.4	1.5 $\pm$ 0.3	1.1 $\pm$ 0.1	4.8	3.4	3.8 $\pm$ 1.1	0.7		
Taurine		2.6 $\pm$ 0.4	5.6 $\pm$ 0.7	1.3 $\pm$ 0.2	1.6 $\pm$ 0.1	2.6	3.5	0.8	1.9		
Asp		0.7 $\pm$ 0.1	1.3 $\pm$ 0.1	0.5 $\pm$ 0.05	0.9 $\pm$ 0.1	1.3	0.6	1.0 $\pm$ 0.2	0.4		
Thr		0.4 $\pm$ 0.1	1.2 $\pm$ 0.1	0.4 $\pm$ 0.05	0.5 $\pm$ 0.1	0.9	0.8	1.3 $\pm$ 0.2	0.3		
Ser		0.3 $\pm$ 0.05	0.9 $\pm$ 0.1	0.3 $\pm$ 0.05	0.4 $\pm$ 0.1	0.4	0.4	0.9 $\pm$ 0.1	0.3		
Pro		0.9 $\pm$ 0.1	2.7	0.4	1.2 $\pm$ 0.1	0.7	Trace	0.8 $\pm$ 0.2	N.D.		
Glu		4.8 $\pm$ 0.2	7.9 $\pm$ 0.2	3.2 $\pm$ 0.2	4.9 $\pm$ 0.2	4.4	4.5	7.7 $\pm$ 0.9	2.1		
Gln		0.7 $\pm$ 0.1	0.4 $\pm$ 0.1	1.1 $\pm$ 0.3	0.9 $\pm$ 0.1	1.5	1.9	1.2 $\pm$ 0.2	0.6		
Gly		2.3 $\pm$ 0.1	9.9 $\pm$ 0.5	3.7 $\pm$ 0.5	5.1 $\pm$ 0.3	3.3	2.3	3.1 $\pm$ 0.3	0.8		
Ala		1.1 $\pm$ 0.1	1.5 $\pm$ 0.1	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1	1.2	0.8	1.9 $\pm$ 0.3	0.2		
Val		Trace	0.2	Trace	Trace	0.2	0.1	0.3 $\pm$ 0.1	Trace		
Met		Trace	Trace	Trace	Trace	0.05	0.1	Trace	Trace		
Ile		Trace	Trace	Trace	Trace	0.1	Trace	0.1	Trace		
Leu		0.1 $\pm$ 0.03	0.2	Trace	0.1 $\pm$ 0.03	Trace	0.1	0.3 $\pm$ 0.05	0.1		
Tyr		0.1 $\pm$ 0.03	Trace	Trace	Trace	0.2	Trace	0.2 $\pm$ 0.05	Trace		
Phe		Trace	Trace	Trace	Trace	0.1	Trace	0.1 $\pm$ 0.05	Trace		
γ -Aminobutyric acid		0	0	Trace	0	0	0	Trace	Trace		
NH ₃		0.8 $\pm$ 0.1	3.3 $\pm$ 0.3	1.2 $\pm$ 0.4	0.6 $\pm$ 0.1	1.3	1.0	1.4 $\pm$ 0.3	2.0		
Lys		0.3 $\pm$ 0.1	0.6	0.2 $\pm$ 0.05	0.2 $\pm$ 0.05	0.3	0.2	0.3 $\pm$ 0.1	0.2		
His		Trace	0.2	Trace	Trace	0.1	0.1	0.2 $\pm$ 0.05	Trace		
Arg		0.2 $\pm$ 0.05	0.3	Trace	0.1	0.2	Trace	0.2 $\pm$ 0.05	Trace		
GSH+GSSG		4.1 $\pm$ 0.1	10.6 $\pm$ 0.5	5.0 $\pm$ 0.1	4.7 $\pm$ 0.2	5.6	3.0	5.1 $\pm$ 0.5	1.9		
Total		20.6	51.2	20.6	23.3	29.3	22.8	30.7	11.5		
Lactate		1.0 $\pm$ 0.1	1.3 $\pm$ 0.3	1.4 $\pm$ 0.1	0.9 $\pm$ 0.1	—	1.2	—	—		

from guinea pigs, rabbits, chickens and frogs and frozen in liquid N₂. Testes were obtained surgically from anaesthetized (Surital; Parke, Davis, Ann Arbor, Mich., U.S.A.) cats. In all cases testes were decapsulated after freezing, before processing. Blood was collected from the decapitated animals into beakers containing heparin (approx. 0.2mg/ml of blood).

Isolation, assay and degradation of amino acids

The amino acids were isolated from acid-soluble extracts of frozen testes by methods described by O'Neal & Koeppe (1966), except that glutamine was hydrolysed enzymically (Mushahwar & Koeppe, 1972). Lactate was isolated from rat testes as previously described for the isolation of brain lactate (Minard & Mushahwar, 1966). The free amino acid concentrations of testicular tissue of the various animals were measured on a Technicon amino acid Analyzer essentially according to the method of Stein & Moore (1954).

Samples of glutamate isolated from the testes of rats 1 and 2, 5 and 7, and 16 and 17 (see Tables 3 and 5) were pooled separately, diluted with carrier, degraded (Hill *et al.*, 1958) and assayed for ¹⁴C (Koeppe & Hill, 1955).

Blood, brain and testes extracts were assayed for lactate content according to the method of Hohorst (1963). Glucose of blood and testes was determined by the Glucostat method (Sigma Chemical Co., St. Louis, Mo., U.S.A.). Pyruvate carboxylase activity of rat testes was assayed essentially according to the radioactivity method of Keech & Utter (1963).

Measurement of radioactivity

Exhaled ¹⁴CO₂ was trapped in NaOH and assayed with a vibrating-reed electrometer (Koeppe & Hill, 1955). The ¹⁴C content of the isolated testes or brain metabolites and of perchloric acid filtrates of blood, brain and testes was measured in a Packard Tri-Carb liquid-scintillation spectrometer by using Bray's (1960) solution as scintillator.

Materials

[1-¹⁴C]Ethanol (3.1mCi/mmol) was purchased from New England Nuclear Corp. (Boston, Mass., U.S.A.). [1-¹⁴C]Acetate (Na⁺ salt, 22mCi/mmol), and D-[2-¹⁴C]glucose (3mCi/mmol) were purchased from Amersham/Searle (Chicago, Ill., U.S.A.). Glutaminase (grade IV), lactate dehydrogenase, glucose oxidase and L-lactate were purchased from Sigma Chemical Co. GSH and GSSG, taurine and phosphorylethanolamine were obtained from Calbiochem (Los Angeles, Calif., U.S.A.). Pyrazole was purchased from Aldrich Chemical Co. (Milwaukee,

Wis., U.S.A.) and insulin was purchased from Eli Lilly and Co. (Indianapolis, Ind., U.S.A.).

Results and Discussion

Free amino acids in testes of different species

The amino acid concentration results are presented in Table 1. There are several noteworthy points. The total free amino acid concentration of gerbil testes is almost fivefold that of frog testes. Thus the water-conserving situation in the gerbil results in a very high free amino acid concentration whereas the more aqueous environment of the frog has a reverse effect. Free amino acid concentrations in the testes of other animals studied were quite similar, intermediate between the gerbil and frog.

The extract from guinea-pig testes always showed a significant ninhydrin peak at a time corresponding to that at which the γ -aminobutyric acid is eluted from the standard amino acid assay column. However, to date no further identification of this peak has been undertaken and we can only say tentatively that guinea-pig testes contain significant amounts of γ -aminobutyric acid.

When compared to that of brain tissue, the glutamate concentration of testes is 50–75% lower. Also the amount of glutamine in testes is much lower than that of brain. However, the glutathione content of testes is severalfold higher than that of rat brain (Minard & Mushahwar, 1966; O'Neal & Koeppe, 1966). This fact coupled with the high glycine content (particularly in the gerbil) suggest the possibility that the amino acid transport system involving glutathione, as proposed by Orlowski & Meister (1970), may be present in testes.

Effects of insulin on free amino acids of testes

Table 2 presents the more striking changes that occurred in the free amino acid concentration of testes of rats treated with large doses of insulin or ammonium acetate. Severe hypoglycaemia in rats results in a marked decrease in brain glutamate and glutamine, with a corresponding increase in aspartate (Dawson, 1950; De Ropp & Snedecker, 1961; O'Neal & Koeppe, 1966). Hypoglycaemia also results in a considerable decrease in the glutamate content of rat testes (Table 2) with some increase in aspartate. However, in the case of testes, glutamine content is increased rather than decreased. The striking decrease in alanine content of testes (Table 2) may be interpreted as a use of this amino acid as an energy source during the hypoglycaemic situation, the amino nitrogen being converted into the amide of glutamine. As in brain, lethal doses of ammonium acetate cause a very rapid increase in glutamine content of testes (Table 2).

Table 2. *Effects of insulin and ammonium acetate treatment on some free amino acid concentrations ($\mu\text{mol/g}$ wet wt. of rat testes*

Two of the insulin-treated animals received 150 units of insulin/kg body wt. 150 min before death. The other four animals required a second dose of insulin to induce severe hypoglycaemic shock. At death the blood sugars of all insulin-treated rats were $<30\text{ mg}/100\text{ ml}$. Rats receiving ammonium acetate ($10.8\text{ mmol}/\text{kg}$ body wt.), were near death when killed 7–8 min after treatment. The insulin animals only, were killed by decapitation (for blood collection) before freezing in liquid N_2 ; others were frozen *in situ*. The number of animals used are given in parentheses. * Values significantly different from the control ($P < 0.01$).

Amino acid	Amino acid concentration ($\mu\text{mol/g}$ wet wt.)		
	Control (6)	+Insulin (6)	+Ammonium Acetate (2)
Ala	1.1 ± 0.1	$0.4 \pm 0.1^*$	1.2
Asp	0.7 ± 0.1	0.9 ± 0.1	0.5
Glu	4.8 ± 0.2	$3.9 \pm 0.2^*$	5.1
Gln	0.7 ± 0.1	$1.5 \pm 0.1^*$	3.3
Thr	0.4 ± 0.1	$0.8 \pm 0.1^*$	1.0
Lys	0.3 ± 0.1	0.5 ± 0.1	0.6
NH_3	0.8 ± 0.1	0.9 ± 0.1	10.0

Labelling of amino acids in testes after injection of $[1-^{14}\text{C}]$ acetate or $[1-^{14}\text{C}]$ ethanol

Table 3 presents the results for radioactivity in the amino acids of rat testes after intraperitoneal injection of either $[1-^{14}\text{C}]$ acetate or $[1-^{14}\text{C}]$ ethanol. As expected, pyrazole, a potent inhibitor of ethanol oxidation (Lester & Benson, 1970; Mushahwar & Koeppe, 1972) markedly decreases the formation of $^{14}\text{CO}_2$ and incorporation of label into testes amino acids (rats 9 and 10). These results suggest, but do not prove, that pyrazole enters the testes (Mushahwar & Koeppe, 1972), and inhibits ethanol oxidation *in situ*. Except for the pyrazole-treated rats the results obtained from the two sets of animals are very similar. For example, the specific radioactivity results from rats 1 and 2 are comparable with those obtained from rats 5 and 6. Also, the addition of 0.5 mmol of carrier acetate (rats 3 and 4) or carrier ethanol (rat 7) did not decrease the specific radioactivity observed in testes amino acids.

Thus it is clear from the results presented in Table 3 that testes are capable of metabolizing both acetate and ethanol. Based on these results, one would predict that testes have small but detectable amounts of alcohol dehydrogenase, a situation already established for rat brain (Raskin & Sokoloff, 1970). One might argue that testes did not metabolize ethanol but rather oxidized acetate formed from ethanol in other tissues. However, the fact that 0.25 mmol of carrier acetate (rat 8) had no effect on the labelling of testes amino acids from $[1-^{14}\text{C}]$ ethanol negates this argument.

The compartmentation of glutamate and glutamine in brain is well documented (Waelsch *et al.*, 1964; O'Neal & Koeppe, 1966; and for a review see Berl &

Clarke, 1969). Labelled acetate or ethanol always results in brain glutamine of substantially higher specific radioactivity than brain glutamate (O'Neal & Koeppe, 1966; Berl & Clarke, 1969; Mushahwar & Koeppe, 1972). Such compartmentation does not exist in testes since without exception (Table 3) labelled acetate or ethanol yields testes glutamate of considerably greater specific radioactivity than testes glutamine.

Labelling of amino acids in testes after injection of $[2-^{14}\text{C}]$ glucose

Radioactivity results in rat testes following administration of $[2-^{14}\text{C}]$ glucose are presented in Table 4. Contrary to some studies *in vitro* (Hollinger & Davis, 1968), after injection of $[^{14}\text{C}]$ glucose *in vivo* only a rather small percentage of the ^{14}C of testes was found in lactate. It will be noted that 10 min after administration of glucose the specific radioactivities of the testes amino acids are much lower than those observed (Table 3) after administration of either labelled acetate or ethanol. However, if 30 or 60 min elapse between administration of the isotope and death of the animal, then a much higher percentage of the ^{14}C in testes resides in the amino acids and the specific radioactivities of the latter are comparable with those seen after the administration of ethanol or acetate. The lag period for incorporation of ^{14}C from labelled glucose into glutamate is much shorter in brain. Within 2 min after the intravenous administration of labelled glucose, more radioactivity resides in brain glutamate than in brain glucose (Cremer, 1970). The striking difference between testes as compared with brain of the rate of incorporation of label from glucose into the amino acids related to the tri-

Table 3. Radioactivity in rat testes, exhaled CO₂ and certain testes amino acids 10min after intraperitoneal administration of [1-¹⁴C]acetate or [1-¹⁴C]-ethanol

For details see the text. The results for ¹⁴C in testes refer to acid-soluble material. Specific radioactivities were: [1-¹⁴C]acetate, 22mCi/mmol; [1-¹⁴C]-ethanol, 3.1 mCi/mmol.

Rat no.	Treatment	Exhaled ¹⁴ CO ₂ (% of dose)	¹⁴ C (nCi/g)	Amino acids (% of acid-soluble ¹⁴ C)	Specific radioactivity (nCi/μmol)		
					Glu	Gln	Asp
1	[1- ¹⁴ C]Acetate (14.3 μCi)	15.7	23	50	2.3	1.3	1.3
2	[1- ¹⁴ C]Acetate (14.3 μCi)	9.7	36	39	3.4	1.7	1.1
3	[1- ¹⁴ C]Acetate (24 μCi) plus 0.5 mmol of acetate	2.0	56	14	2.0	0.5	0.4
4	[1- ¹⁴ C]Acetate (24 μCi) plus 0.5 mmol of acetate	2.6	35	22	1.2	0.3	0.6
5	[1- ¹⁴ C]Ethanol (15.5 μCi)	10.4	16	55	1.8	0.6	0.8
6	[1- ¹⁴ C]Ethanol (15.5 μCi)	7.1	29	62	5.0	0.6	1.9
7	[1- ¹⁴ C]Ethanol (15.5 μCi) plus 0.5 mmol of ethanol	15.7	18	39	1.3	0.3	0.7
8	[1- ¹⁴ C]Ethanol (15.5 μCi) plus 0.25 mmol of acetate	4.0	21	60	2.4	0.7	1.3
9	[1- ¹⁴ C]Ethanol (15.5 μCi) 30min after pyrazole treatment	0.6	19	4	0.1	0.05	0.02
10	[1- ¹⁴ C]Ethanol (15.5 μCi) 30min after pyrazole treatment	0.3	27	2	0.1	0.03	0.08

Table 4. Radioactivity in rat testes, exhaled CO₂ and certain testes amino acids and lactate at various times after intraperitoneal administration of 9.6 μCi of D-[2-¹⁴C]glucose

For details see the text. The unutilized glucose was determined as described by Minard & Mushahwar (1966)

Rat no.	Time (min)	Exhaled ¹⁴ CO ₂ (% of dose)	¹⁴ C in testes (nCi/g)	Unutilized glucose (% of acid-soluble ¹⁴ C)	Lactate in testes (% of acid-soluble ¹⁴ C)	Amino acids in testes (% of acid-soluble ¹⁴ C)	Specific radioactivity (nCi/μmol)		
							Glu	Gln	Asp
11	10	0.4	31	—	—	3.4	0.21	0.05	0.16
12	10	0.4	22	—	—	3.3	0.16	0.03	0.14
13	10	1.2	25	81	10	1.6	0.09	—	0.10
14	10	—	31	68	14	—	—	—	—
15	10	—	48	71	13	—	—	—	—
16	30	4.5	27	54	9	17.5	1.20	0.32	0.56
17	30	13.9	30	61	6	20.1	1.26	0.33	0.93
18	60	31.4	18	43	7	27.4	1.74	0.36	0.46
19	60	21.6	24	35	7	37.3	1.89	1.28	0.99
20	60	17.8	23	41	6	31.7	1.92	0.44	0.89

Table 5. *Distribution of radioactivity in glutamic acid of rat testes*

Glutamic acid of testes was pooled, diluted with carrier, crystallized, degraded and assayed for ^{14}C (see the text).

Compound administered	Rat no. (see Tables 3 and 4)	% of total radioactivity incorporated in	
		C-1	C-5
[1- ^{14}C]Acetate	1 and 2	10.4	83.2
[1- ^{14}C]Ethanol	5 and 7	5.6	86.1
D-[2- ^{14}C]Glucose	16 and 17	23	67.7

carboxylic acid cycle cannot be explained by differences in the size of the glucose pools of these two tissues. In fact, these pools are of similar magnitude [brain, $0.45\mu\text{mol/g}$ wet wt. (Balázs & Haslam, 1965); testes, $0.7\mu\text{mol/g}$ (determined by us in agreement with Professor L. L. Ewing, personal communication)]. Thus the results presented in Table 4 seem to indicate that testes absorb blood glucose quite rapidly but oxidize this glucose more slowly than does brain.

Even if one considers the dilution of administered [2- ^{14}C]glucose by blood glucose, the results presented in Tables 3 and 4 suggest that testes oxidize acetate or ethanol at rates comparable with that for glucose. Since it is not known if triglycerides or fatty acids of blood are satisfactory energy substrates for testes, the question of whether damage to testes caused by hypoglycaemia results from an energy-deficient situation in the testes cannot be answered at the present time.

Distribution of radioactivity in glutamate after administration of various precursors

The distribution of radioactivity in glutamate isolated from testes after administration of the various labelled precursors is presented in Table 5. The very high labelling of C-5 following administration of either [1- ^{14}C]acetate or [1- ^{14}C]ethanol indicates that the ^{14}C in testes did not result from prior conversion of these compounds into blood glucose in the liver. Such a route would result in [3,4- ^{14}C]glucose and [1- ^{14}C]glutamate in the testes (Mushahwar & Koeppe, 1972). The results with [2- ^{14}C]glucose where the C-5/C-1 ratio is three and the radioactivity in C-5 approaches 70% are noteworthy. No other tissue studied to date, except brain (O'Neal & Koeppe, 1966), converts [2- ^{14}C]glucose into glutamate with this high percentage of the ^{14}C in C-5. This finding indicates that testis, like brain, converts most of its pyruvate (lactate) into acetate before metabolism in the tricarboxylic acid cycle. A similar finding is that testis, like brain, has much less CO_2 -fixation capacity (pyruvate carboxylase activity) than does liver or kidney (Keech & Utter, 1963). We find the

pyruvate carboxylase activity in rat testes to be approx. $0.13\mu\text{mol/min}$ per g, a value very close to that for rat brain (Salganicoff & Koeppe, 1968).

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