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Octopine Metabolism in the Cuttlefish, *Sepia officinalis*: Octopine Production by Muscle and Its Role as an Aerobic Substrate for Non-Muscular Tissues

Kenneth B. Storey* and Janet M. Storey
The Laboratory, Citadel Hill, Plymouth PL1 2PB, England

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Summary. 1. The metabolism of the glycolytic end product, octopine, was investigated in vivo in the cuttlefish, *Sepia officinalis*. Octopine was the major mantle muscle end product produced during hypoxia, exhaustive swimming, or exhaustive swimming followed by hypoxia (muscle octopine rose from 0.2 to 3.7, 8.6, and 13.4 μmol/g wet wt. respectively). Octopine concentration was inversely correlated with muscle glycogen and arginine phosphate concentrations and these substrates were almost completely depleted after swimming to exhaustion. Alanine, α-glycerophosphate, pyruvate, and malate were other, minor end products.

2. Blood octopine (0.02 μmol/ml at rest), pyruvate, and alanine concentrations were elevated during hypoxia and during recovery from hypoxia or exercise but not during exercise itself. Maximal blood octopine concentrations were 8-fold higher than resting levels and blood octopine appeared to be derived from the release of muscle octopine into the bloodstream.

3. ^{14}C-A-octopine (radiolabelled in the arginine moiety: N^2-(1-carboxyethyl)[U-^{14}C]L-arginine) was administered intravenously and tissue uptake patterns showed that mantle muscle was relatively poor at the uptake of blood ^{14}C-A-octopine while brain and ventricle rapidly concentrated the compound. Parallel experiments in which [U-^{14}C]D-glucose or [U-^{14}C]L-arginine were administered showed that there are distinct tissue specific uptake patterns for each of the three radiolabelled compounds.

4. The tissue breakdown of ^{14}C-A-octopine taken up from the blood to form ^{14}C-arginine was found to be 0, 6, 32 and 20% respectively for mantle muscle, gill, ventricle, and brain. When delivered by specific injection into mantle muscle or brain, ^{14}C-A-octopine oxidation was 5% and 40% respectively after 20 min under resting, aerobic conditions.

5. The data indicate that while mantle muscle readily produces octopine as a glycolytic end product, the tissue has little capacity for the oxidation of octopine. Muscle octopine appears to be released into the bloodstream and can be readily taken up by other tissues. The pyruvate moiety of octopine could be oxidized as an aerobic substrate by the Krebs cycle in tissues such as brain and ventricle with the arginine moiety being recycled to the muscle. The possible existence of a modified Cori cycle, to make use of octopine as a gluconeogenic substrate, is discussed.

Introduction

Anaerobic glycolysis in the mantle muscle of cephalopod molluscs, and in the muscles of some bivalves (Regnouf and Thoai, 1970), results in the production of octopine, (N^2-(l-carboxyethyl)L-arginine), an end product analogous in function to lactate which is produced via the octopine dehydrogenase reaction: pyruvate + arginine + NADH $\rightleftharpoons$ octopine + NAD^+. While octopine synthesis in working muscle has been studied in some detail (Hochachka et al., 1977); Grieshaber and Gade, 1976), the subsequent metabolic fate of muscle octopine accumulated during work has not yet received attention. In vertebrate systems, muscle lactate is released into the bloodstream at the cessation of work and oxidized as an aerobic substrate by tissues such as heart and brain or reconverted to glucose by gluconeogenic reactions in liver and kidney. An analogous situation could be hypothesized for the catabolism of muscle octopine by cephalopod

* Permanent address: Dept. of Biology, Carleton University, Ottawa, Ontario, Canada K1S 5B6

Abbreviations: *^{14}C-A-octopine*, octopine radiolabelled in the arginine moiety; N^2-(1-carboxyethyl) [U-^{14}C)-L-arginine; *^{14}C-P-octopine*, octopine labelled in the pyruvate moiety, N^2-(1-[2-^{14}C] carboxyethyl) L-arginine

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tissues. The tissue distribution of octopine dehydrogenase isozymes strongly supports such a hypothesis (Storey, 1977; Storey and Storey, 1979). The muscle form of octopine dehydrogenase is kinetically similar to the M isozyme of lactate dehydrogenase and functions in vivo in the rapid synthesis of octopine under conditions of rising muscle pyruvate and arginine concentrations (Fields et al., 1976). The brain specific isozyme of octopine dehydrogenase, however, displays kinetic properties, including pyruvate substrate inhibition, which indicate a function analogous to that of H type lactate dehydrogenase (Storey and Storey, 1979). Thus brain, and other tissues displaying this isozyme of octopine dehydrogenase, could be poised to make effective use of octopine as an aerobic substrate.

In the present study we have combined an investigation of octopine production in the mantle muscle of the cuttlefish, *Sepia officinalis*, with experiments designed to test the oxidation of ^{14}C-labelled octopine by *Sepia* tissues.

Materials and Methods

Animals, Chemicals, and Radiochemicals

Cuttlefish, *Sepia officinalis* (13–21 cm long, 75–135 g), of both sexes, were held in running seawater for at least 2 days before use and fed crabs. All biochemicals and coupling enzymes were purchased from Sigma Chemical Co. Radiochemicals, [U-^{14}C]L-arginine (287 mCi/mmol), sodium [3-^{14}C]pyruvate (21 mCi/mmol), and [U-^{14}C]D-glucose (13.9 mCi/mmol) were purchased from New England Nuclear Corp.

Preparation of ^{14}C-Octopine

Octopine radiolabelled in the arginine moiety, ^{14}C-A-octopine, and octopine radiolabelled in the pyruvate moiety, ^{14}C-P-octopine, were prepared enzymatically using either ^{14}C-arginine or ^{14}C-pyruvate as precursors. Octopine dehydrogenase was purified from scallop adductor muscle by the method of Fields et al. (1976). The reaction conditions used for the synthesis of ^{14}C-A-octopine were: 10 µmol pyruvate, 3.5 µmol NADH, 60 µCi ^{14}C-arginine (0.24 µmol arginine), 100 µl purified octopine dehydrogenase preparation, and 20 mM imidazole, pH 7.0 in a total volume of 3 ml. After a 30 min incubation period additional non-radioactive arginine (2.5 µmol in 3 additions at 20 min intervals) was added. For the synthesis of ^{14}C-P-octopine, the reaction conditions were: 30 µmol arginine, 15 µmol NADH, 50 µCi ^{14}C-pyruvate (3 µmol pyruvate), 100 µl purified enzyme, and 20 mM imidazole buffer, pH 7.0 in a total volume of 3 ml. The reaction was conducted similarily to that for the ^{14}C-A-octopine preparation and an additional 8 µmol non-radioactive pyruvate was added in 3 additions. The enzymatic reaction was stopped by the addition of 8 ml hot ethanol. Amberlite IR 120 column chromatography was used to purify ^{14}C-octopine. Ethanol extracts were first passed down the Na^+ form of the column at pH 7.0 which binds arginine but not octopine (Hiltz and Dyer, 1971). The octopine-containing eluant was then applied to the column at pH 2.0 which binds octopine but not pyruvate. Octopine was eluted from this column in 2 N NH_4OH and concentrated using a rotary evaporator. ^{14}C-octopine was then made up in 5 ml 0.01 N HCl and stored frozen. Conversion of radiolabelled precursors to ^{14}C-octopine was 91% and 92% complete for the ^{14}C-arginine and ^{14}C-pyruvate reactions, respectively. Paper chromatography of ^{14}C-octopine after column chromatography purification and concentration showed a 99% radiochemical purity. Liquid scintillation spectrometry and enzymatic assay of octopine content (see assay below) were combined to measure specific activity. Final specific activities were 8.2 mCi/mmol for ^{14}C-A-octopine and 3.2 mCi/mmol for ^{14}C-P-octopine.

Mantle Muscle Metabolite Concentrations

Metabolite concentrations in mantle muscle were measured under 2 control conditions: resting in aerated water and ethanol anaesthesia, and 4 experimental conditions: mild hypoxia, severe hypoxia, exhaustive swimming, and exhaustive swimming followed by recovery in hypoxic water. To obtain control muscle samples from animals resting in aerated water, animals were rapidly netted and plunged into liquid nitrogen followed by dissection of pieces of frozen muscle. In all other cases, animals were killed rapidly by severing the nerve cord at the neck, and muscle samples rapidly excised and frozen in liquid nitrogen. Ethanol anaesthesia was accomplished by slowly mixing 95% ethanol into seawater. Animals lose their equilibrium in about 2 min and can then be easily handled, in or out of water, without struggle. The effects of ethanol are freely reversible upon removal to fresh seawater. Mild hypoxia was induced by placing individual *Sepia* in small containers of seawater and allowing the animals to deplete the oxygen content by respiration alone. Approximately one hour was required for an individual to lower the P_{O_2} of the water from 130 to 30 mm Hg and the experiment was terminated when 30 mm Hg was reached. Severe hypoxia was induced by bubbling the seawater in a small container with nitrogen gas after first allowing 30 min for an individual animal to lower the P_{O_2} by respiration alone. Bubbling with nitrogen gas reduced the P_{O_2} to 10 mm Hg and animals were exposed to 30 min of this O_2 tension. In both types of hypoxia, animals showed signs of distress. Excercise experiments were accomplished by forcing individuals to swim until exhausted in a large, well-aerated seawater tank. Where hypoxia was given after exercise, the exercise-exhausted animal was transferred to a tank which had been previously bubbled with nitrogen gas to a P_{O_2} of approximately 10 mm Hg. Animals were exposed to this O_2 tension for 30 min.

Muscle samples were stored in liquid nitrogen until use. Samples of muscle were powdered under liquid nitrogen using a mortar and pestle, weighed, and homogenized in 5 volumes ice-cold 6% perchloric acid containing 1 mM EDTA using a Polytron homogenizer. An aliquot of homogenate was then removed for glycogen determination and the remaining homogenate centrifuged at $10{,}000 \times g$ for 10 min. The supernatant was removed and neutralized by addition of 1.5 M KOH, 0.4 M imidazole, 0.3 M KCl. Precipitated $KClO_4$ was removed by centrifugation. Neutralized extracts were frozen in liquid nitrogen until analysis.

Muscle metabolite concentrations were measured spectrophotometrically in pyridine nucleotide-coupled enzymatic assays. ATP, glucose, pyruvate, α-glycero-P, alanine, malate, and lactate were determined using the assays of Lowry and Passonneau (1972). Glycogen was determined as glucose after hydrolysis with amyloglucosidase (Keppler and Decker, 1974). Arginine phosphate was determined as described by Storey and Storey (1978). Octopine and arginine were measured using purified scallop octopine dehydrogenase and assay conditions: 0.5 mM NAD^+ and 100 mM Tris buffer, pH 8.5 for octopine, and 3 mM pyruvate, 0.15 mM NADH, and 50 mM imidazole buffer, pH 7.0 for arginine.

Blood Metabolite Levels

In the experiments described above, blood samples were withdrawn by syringe from the vena cava at the time of death. In separate experiments, animals were exposed to conditions of severe hypoxia and exhaustive exericse identical to those described previously and then transferred to normoxic seawater for 5 min of recovery. Animals were then killed and blood samples taken. Blood was deproteinized in ice-cold perchloric acid/EDTA and neutralized samples prepared as described above. Alanine and pyruvate were determined by the assays of Lowry and Passonneau (1972). Addition of 5 mM EDTA to the assay for octopine was necessary for the measurement of blood octopine levels (J. Fields, personal communication).

Radiotracer Studies

A) Injection into the Vena Cava. To administer radiotracer, animals were briefly held out of water, the mantle cavity held open, and injection made into the anterior vena cava. Animals were then returned to normoxic seawater and given 20 min of rest. Animals were killed by severing the nerve cord, a blood sample taken from the vena cava, and tissues were rapidly excised and frozen in liquid nitrogen. Radiotracers were mixed with a small volume of phosphate buffer, pH 7.0 for injection. The doses used were: ^{14}C-A-octopine, 0.2 ml containing 0.7 μCi and 0.99 μmol octopine; ^{14}C-arginine, 0.1 ml seawater containing 2 μCi ^{14}C-arginine and 0.10 μmol non-radioactive arginine added; ^{14}C-glucose, 0.1 ml seawater containing 2 μCi ^{14}C-glucose and 0.14 μmol glucose.

Perchloric acid extracts of blood and tissues were made as described for the analysis of metabolite levels. Total radioactivity in each tissue was determined by counting aliquots of the tissue extracts using Amersham PCS liquid scintillation fluid. Duplicate samples of mantle muscle were prepared and muscle radioactivity determined from the average.

The composition of tissue radioactivity was determined by descending paper chromatography on Whatman No. 1 paper with the solvent system of Crowley et al. (1963). This system effectively separates arginine, octopine, arginine phosphate, and glucose with R_f's of 0.67, 0.46, 0.16, and 0.30, respectively. Radioactive peaks were determined by counting 1 cm strips of the dried chromatograms by liquid scintillation spectrometry.

B) Specific Site Injections. ^{14}C-A-octopine (0.1 μCi), ^{14}C-P-octopine (0.3 μCi) was injected into specific sites in mantle muscle, hepatopancreas, or brain. After injection, animals were allowed 20 min rest in aerated water after which they were killed, blood samples taken, and the injected tissues excised and frozen in liquid nitrogen. Perchloric acid extracts of blood and tissues were made, total tissue radioactivity determined, and chromatography of blood and tissue extracts performed.

Results

Muscle Metabolite Concentrations Under Resting, Hypoxic, and Exercise Conditions

Table 1 shows the effects of experimental stresses on the levels of some intermediary metabolites in *Sepia* mantle muscle. Two control conditions were used. Muscle was sampled from resting animals quick-frozen whole in liquid nitrogen or muscle samples were excised from live, ethanol anaesthetized animals and quickly frozen in liquid nitrogen. Both types of controls produced essentially the same results with no significant difference between the two treatments for any of the metabolites measured. In Table 1, the results from 4 animals frozen whole and 3 animals ethanol anaesthetized have been averaged. Ethanol anaesthesia, therefore, appears to be an excellent method for use in experiments requiring manipulation of resting animals without struggle. The qualitative effects of hypoxia or exercise on mantle muscle metabolism

Table 1. Some metabolite levels in the mantle muscle of *Sepia officinalis* under conditions of rest, hypoxia, and exercise

Metabolite	Experimental condition				
	Control resting (7)	Mild hypoxia (3)	Severe hypoxia (4)	Exhaustive exercise (4)	Exercise + hypoxia (3)
ATP	8.68 ± 0.66	7.89 ± 0.27[a]	4.47 ± 0.28	2.15 ± 0.69	2.44 ± 1.22
Arginine phosphate	33.62 ± 1.66	21.40 ± 3.30	18.80 ± 3.00	3.76 ± 0.63	0.80 ± 0.40
Arginine	29.60 ± 0.60	35.90 ± 2.43	37.50 ± 2.47	45.30 ± 1.64	38.60 ± 1.35
Octopine	0.17 ± 0.12	3.72 ± 1.70	3.64 ± 1.13	8.59 ± 0.71	13.43 ± 0.42
Sum: arginine-P + arginine + octopine	63.4	61.0	59.9	57.7	52.8
Glycogen (as glucose)	23.70 ± 1.61	13.80 ± 2.30	2.15 ± 0.85	4.50 ± 2.20	1.90 ± 1.20
Glucose	0.72 ± 0.07	2.70 ± 0.36	2.56 ± 0.55	1.25 ± 0.21[b]	1.84 ± 0.60[b]
Pyruvate	0.09 ± 0.01	0.30 ± 0.05	0.16 ± 0.02	0.76 ± 0.08	0.26 ± 0.02
Alanine	4.77 ± 0.88	–	8.63 ± 0.85[b]	10.00 ± 1.50[b]	9.56 ± 1.06[b]
α-Glycerophosphate	0.33 ± 0.02	–	0.79 ± 0.18	1.36 ± 0.07	1.16 ± 0.15
Malate	0.04 ± 0.01	–	0.16 ± 0.04	0.20 ± 0.03	0.19 ± 0.04

Muscle metabolite levels are given as μmol/g wet weight ± s.e.m., *n* for each condition is given in brackets. Significant difference from control levels was tested using the Student's *t*-test (two-tailed)

[a] Not significantly different from control

[b] Significantly different, $P < 0.02$. All other results are significantly different from controls, $P < 0.01$. Experimental conditions are discussed in detail in Materials and Methods

(loss of energy stores, build-up of anaerobic and products) are similar but, quantitatively, burst swimming was a far greater stress upon muscle reserves than was an hour or more of hypoxia.

Arginine phosphate stores in mantle muscle are large and are rapidly depleted during hypoxia or exercise stress. However, despite the mobilization of arginine phosphate, ATP concentrations fell under conditions of severe hypoxia or exercise. ATP levels in exercised muscle (and also in exercise + hypoxia animals) fell to approximately one quarter of the concentration in resting muscle. This highly labile nature of the ATP pool in *Sepia* muscle is also seen in other cephalopod muscles (Storey and Storey, 1978) although it is not generally the case in vertebrate muscles. In response to the hydrolysis of arginine phosphate during hypoxia or exercise stress, muscle free arginine concentrations increased somewhat. However, most of the decrement in the arginine phosphate pool appeared as an increase in muscle octopine concentration. The total pool of arginine phosphate + arginine + octopine in muscle showed a tendency to decrease with increasing severity of the stress and was decreased by 17% in muscle from animals first exercised and then exposed to hypoxic water. A release of octopine produced in mantle muscle into the bloodstream may be indicated.

The primary substrate of mantle muscle appears to be glycogen and these reserves are largely depleted by hypoxia or exhaustive exercise. Indeed the physical exhaustion of the animals after the burst swimming episodes can be correlated with an almost complete depletion of the anaerobic energy sources of the muscle: glycogen and arginine phosphate. While the major end product of glycogenolysis in mantle muscle is octopine, significant increases in the amounts of pyruvate, α-glycerophosphate, malate, and alanine were also found in the hypoxic or exercised muscle. Alanine, in particular, increases in concentration 4–5 μmol/g wet wt. and may be the important immediate end product during the transition from aerobic to anaerobic metabolism as its concentration stabilizes while that of octopine continues to increase in proportion to glycogen breakdown. Lactate concentration in mantle muscle was less than 0.05 μmol/g wet wt. under all conditions and no build-up of muscle lactate was detected under either hypoxic or exercise conditions. Muscle glucose levels were also elevated during hypoxia or exercise stress, an indication perhaps of an increased uptake (and utilization) of blood glucose under these conditions.

Table 2. Some metabolite levels in the blood of *Sepia officinalis* under conditions of rest, hypoxia or exercise stress, and recovery from stress

Experimental condition	Octopine	Pyruvate	Alanine
Resting control (3)	0.02 ± 0.007	0.06 ± 0.008	0.06 ± 0.002
Severe hypoxia (4)	0.08 ± 0.018[a]	0.11 ± 0.010[b]	0.24 ± 0.030[c]
Severe hypoxia + recovery (3)	0.06 ± 0.010[a]	0.08 ± 0.006	0.20 ± 0.035[b]
Exhaustive exercise (4)	0.03 ± 0.002	0.09 ± 0.006[a]	0.09 ± 0.010
Exhaustive exercise + recovery (3)	0.05 ± 0.008[a]	0.11 ± 0.009[b]	0.18 ± 0.030[b]
Exercise + hypoxia (3)	0.16 ± 0.002[c]	0.20 ± 0.030[b]	0.19 ± 0.020[c]

Metabolite concentrations are given as μmol/ml blood ± s.e.m., n is given in brackets. Experimental conditions are given in detail in Materials and Methods. Statistically significant differences from controls were tested using the Student's *t*-test (two-tailed). Results are significantly different from controls at [a] $P<0.05$, [b] $P<0.02$, [c] $P<0.01$

Blood Metabolite Concentrations

Table 2 shows the concentrations of some metabolites in *Sepia* blood under the conditions (ethanol anaesthesia control, hypoxia, exercise) used in the muscle metabolite experiments summarized in Table 1. Additionally, blood levels of metabolites were measured in animals exposed to either hypoxia or exercise followed by 5 min recovery in normoxic water.

Hypoxia resulted in elevated levels of octopine, pyruvate, and alanine in the blood and these metabolites remained elevated during the recovery period. Malate and α-glycerophosphate, which were end products accumulated in the hypoxic muscle (Table 1) were not detected in the blood under any of the conditions tested. Exhaustive exercise failed to produce significant changes in the concentrations of blood octopine, pyruvate, or alanine. During the recovery from exercise, however, the levels of all three of these metabolites rose in the bloodstream. Exhaustive exercise followed by recovery in hypoxic water resulted in the largest increases in blood metabolite levels. In this case, end product release from the exercised muscle may continue but the uptake of these end products by tissues outside the muscle mass may be retarded due to the hypoxic state. Thus higher concentrations of octopine, pyruvate, and alanine accumulate in the blood when the aerobic utilization of these compounds is blocked. Although the overall concentrations of anaerobic end products in *Sepia* blood appear to be relatively low, especially when compared to the blood lactate levels developed during recovery from exercise in vertebrates, the increase in blood

metabolite concentrations between the resting versus the exercise/hypoxia state is large: 8-fold, 3.5-fold, and 3-fold for octopine, pyruvate, and alanine respectively. In a system with a rapid circulation time and a highly efficient tissue uptake of anaerobic end products, large amounts of muscle octopine could be transferred to aerobic tissues capable of oxidizing octopine.

Tissue Uptake of Radiotracers

Uptake of ^{14}C-A-octopine, ^{14}C-arginine, and ^{14}C-glucose by *Sepia* tissues was investigated. The results, summarized in Table 3, show the distribution of radioactivity in selected tissues of *Sepia* after vena cava injection of radiotracer and 20 min at rest in normoxic water. A calculation of the percentage of total radioactivity in each tissue divided by the percentage of the body weight in each tissue is also presented. This ratio indicates the relative effectiveness of each tissue in the uptake of blood octopine, arginine, or glucose. To decrease the possibility of blood radioactivity contamination of tissues, tissues were quickly rinsed in seawater after being excised. Radioactivity remaining in the blood was also measured at the end of each experiment. In many cases, and particularly with respect to the smaller tissues such as brain, ventricle, and branchial heart, the weight of blood which would be needed to account for tissue radioactivity far exceeded the actual tissue weight. Thus the contamination of tissue radioactivity by residual blood radioactivity is thought to be minimal.

While the largest percentage of recovered ^{14}C-A-octopine was found in mantle muscle, mantle was, relative to tissue weight, the least effective tissue in the uptake of ^{14}C-A-octopine. The uptake of ^{14}C-A-octopine by the hepatopancreas is also relatively low when expressed relative to tissue weight but of the three radiotracers used, ^{14}C-A-octopine was the one most readily taken up by hepatopancreas. Ventricle, branchial heart, gill, and brain were all very effective in removing ^{14}C-A-octopine from the blood and, as will be discussed later, these tissues were also the ones which demonstrated the highest capability for the oxidation of ^{14}C-A-octopine.

The uptake pattern of ^{14}C-arginine differed somewhat from that seen for ^{14}C-A-octopine. While the greatest percentage of the ^{14}C-arginine dose was again taken up by muscle, the heart tissues, gill, and brain again accumulated the greater amount of radioactivity proportional to their weight. The hepatopancreas was very poor at the uptake of blood ^{14}C-arginine, a reflection perhaps of the low arginine/arginine phosphate pools in this tissue (approximately 2 μmol/g wet wt. in total; Storey and Storey, unpublished data).

The uptake pattern for ^{14}C-glucose was markedly different from those seen for ^{14}C-octopine or ^{14}C-arginine (Table 3). Mantle muscle took up ^{14}C-glucose much more readily than it did ^{14}C-arginine or ^{14}C-A-octopine. Indeed, a comparison of the perchloric acid soluble radioactivity in mantle muscle before and after treatment with amyloglucosidase (to degrade glycogen) resulted in a 13% increase in muscle radioactivity. A rapid incorporation of blood glucose into muscle glycogen is therefore indicated. The other outstanding feature of ^{14}C-glucose metabolism was the strong uptake of glucose by the brain of *Sepia* indicating that cephalopod brain may be highly dependent upon blood glucose as an energy source. The uptake of ^{14}C-glucose by gill, branchial heart, and ventricle was relatively lower than the uptake by these tissues of either ^{14}C-A-octopine or ^{14}C-arginine. While hepatopancreas uptake of ^{14}C-glucose was also low, 24% the tissue ^{14}C-glucose was found to be

Table 3. Distribution of radioactivity in tissues of *Sepia officinalis* after injection into the vena cava of ^{14}C-A-octopine, ^{14}C-arginine, or ^{14}C-glucose

Tissue	^{14}C-A-octopine		^{14}C-arginine		^{14}C-glucose	
	Tissue ^{14}C (% total)	% total ^{14}C/ % body wt.	Tissue ^{14}C (% total)	% total ^{14}C/ % body wt.	Tissue ^{14}C (% total)	% total ^{14}C/ % body wt.
Mantle muscle	60.0±5.4	0.73	53.1±7.0	0.64	76.5±1.3	1.00
Hepatopancreas	10.0±2.8	0.97	1.5±0.3	0.11	1.8±0.5	0.14
Gill	24.2±3.7	2.57	35.6±3.1	3.88	12.5±1.4	1.60
Ventricle	3.1±0.8	11.46	2.1±0.5	6.30	0.6±0.2	2.30
Branchial heart	3.0±0.6	5.58	2.9±0.8	4.21	0.7±0.1	1.20
Brain	4.5±1.9	3.63	6.6±3.0	9.43	8.1±0.1	6.30

Tissue uptake is given as a percentage of the radioactivity recovered ± s.e.m. for $n=4$ (^{14}C-A-octopine) or $n=2$ (^{14}C-arginine, ^{14}C-glucose) trials. Total radioactivity recovered in these tissues was 80% or greater of the injected dose for octopine and arginine trials, and >93% for glucose trials. The remaining radioactivity was localized in blood, gut, and gonad tissues

incorporated into glycogen. Hepatopancreas may, therefore, be an important tissue in the regulation of blood glucose levels, especially in the disposition of dietary glucose.

Tissue Catabolism of ^{14}C-Octopine

To investigate the catabolism of ^{14}C-A-octopine in vivo, the composition of the radioactive component of the perchloric acid extracts of tissues from animals injected intravenously with ^{14}C-A-octopine was analyzed by paper chromatography. The results are summarized in Table 4. ^{14}C-A-octopine in the mantle muscle was not catabolized to release ^{14}C-arginine. This is a key observation in that it demonstrates that the mantle muscle, under resting conditions, has a very poor capacity for the reversal of the octopine dehydrogenase reaction. Thus despite properties which allow the uptake of octopine into the muscle, the capacity for the endogenous oxidation of octopine produced during muscle work appears to be very limited. Brain, ventricle, and gill, however, showed a significant capacity for the breakdown of ^{14}C-A-octopine. Brain and ventricle, in particular, showed 20 and 32% of tissue ^{14}C-A-octopine, respectively, converted to ^{14}C-arginine during the 20 min incubation period under aerobic conditions. The incorporation of radiolabel into ^{14}C-arginine phosphate was tested for but not found in any of the tissues tested.

^{14}C-A-octopine catabolism by brain and mantle muscle were further tested by direct injection of ^{14}C-A-octopine into these tissues. After direct injection, followed by 20 min at rest in normoxic water, 40% of the injected dose had been converted to ^{14}C-arginine in the brain (Table 4). In mantle muscle, however, only 5% of the injected ^{14}C-A-octopine had been converted to ^{14}C-arginine. Thus the poor capacity for octopine oxidation by mantle muscle is further reinforced. Specific injection of ^{14}C-A-octopine into the hepatopancreas was also performed. While the results indicated a significant production of ^{14}C-arginine, a clear determination of the percentage breakdown of ^{14}C-A-octopine was hampered due to a loss of the normally clean separation of octopine and arginine peaks in hepatopancreas chromatograms. Further investigations of octopine metabolism in the hepatopancreas will be of great interest, particularly with respect to the utilization of octopine as a gluconeogenic substrate by the organ.

Brief investigation was also made of the catabolism of ^{14}C-octopine labelled in the pyruvate moiety. ^{14}C-P-octopine was administered by direct injection into mantle muscle or hepatopancreas followed by 20 min under resting conditions in normoxic water. [3-^{14}C]pyruvate was chosen so as to minimize $^{14}CO_2$ production. Paper chromatography of perchloric acid extracts of ^{14}C-P-octopine-injected muscle showed that 2.5% of the injected dose was localized in a peak corresponding in R_f to that of the carboxylic acids succinate, malate, and α-ketoglutarate. This same peak of radioactivity appeared on chromatograms of hepatopancreas extracts but was somewhat larger, representing 5.6% of the injected dose. Incorporation of ^{14}C into glycogen was not detected in these experiments in either muscle or hepatopancreas.

Table 4. Tissue catabolism of ^{14}C-A-octopine administered by vena cava injection or by specific site injection. Data are per cent conversion of ^{14}C-A-octopine to ^{14}C-arginine

Tissue	Mode of injection	
	Vena cava	Specific site
Mantle muscle	0% (2)	5.0% (3)
Gill	5.8 (2)	
Ventricle	32.0 (2)	
Brain	20.0 (2)	40.0 (3)

^{14}C-A-octopine was administered either via the vena cava or via direct injection into muscle or brain. After 20 min rest under aerobic conditions, tissues were excised and the percentage breakdown of ^{14}C-A-octopine to form ^{14}C-arginine determined by chromatography. The percent ^{14}C-arginine per tissue is given as an average for (*n*) trials

Discussion

Bouts of burst swimming are used by *Sepia* to catch prey or evade predators. This sort of swimming appears to be supported by anaerobic glycolysis: glycogen and arginine phosphate providing the ATP for muscle work and octopine being the product. Indeed the onset of exhaustion after forced burst swimming appears to be due to an almost complete depletion of muscle glycogen and arginine phosphate reserves. Hypoxia resulted in a qualitatively similar activation of anaerobic glycolysis in mantle muscle and a degree of muscle tolerance to hypoxic waters may be important to survival during the summer months when *Sepia* are found in warm, shallow inshore and estuarine waters.

Alanine, pyruvate, α-glycerophosphate, and malate were other minor end products of *Sepia* mantle muscle metabolism and are also products of muscle work in other cephalopod species (Hochachka et al., 1975; Grieshaber and Gäde, 1976). The muscle concentrations of these compounds, while elevated over the control levels, nonetheless remained rela-

tively constant despite the experimental stress while octopine concentrations increased with increasing severity of stress. The synthesis of these four minor products of muscle work appears, therefore, to be linked to the initial onset of glycolytic work, when energy demand exceeds the capacity of aerobic muscle metabolism. Indeed, the accumulation of α-glycerophosphate demonstrates a blockage of the mitochondrial portion of the α-glycerophosphate cycle, the cycle used in cephalopod tissues for the transfer of reducing equivalents from cytoplasm to mitochondrion (Hochachka et al., 1975; Storey and Hochachka, 1975). The extremely low activities of lactate dehydrogenase in *Sepia* mantle muscle (octopine dehydrogenase: lactate dehydrogenase activity ratio = 62 (Storey, 1977)) probably accounts for the failure to detect lactate as an end product in mantle muscle. Octopine, then, is the true or major end product of anaerobic glycolysis and accumulates in proportion to muscular work.

The production of octopine as the end product of glycolytic muscle work in *Sepia* is functionally analogous to the synthesis of lactate in working vertebrate skeletal muscle and serves to maintain redox balance during glycolytic function. The lactate produced during vertebrate muscle work is largely released into the bloodstream at the cessation of muscle work and is taken up as an aerobic substrate by other tissues. Lactate is oxidized by the Krebs cycle in brain or heart or is utilized as a gluconeogenic substrate by liver and kidney cortex (Everse and Kaplan, 1973). Via the Cori cycle, in which muscle lactate is reconverted to glucose in liver and then released into the bloodstream, an effective recycling of carbohydrate substrate to the muscle is accomplished.

The results of the present study indicate that the fate of octopine produced during muscle work is also likely to be catabolism outside of the muscle mass. A decrease in the total pool size of arginine phosphate/arginine/octopine in muscle with increasing severity of stress was found (Table 1) and could indicate a release of octopine from the muscle into the bloodstream. This decrease in pool size was largest during the recovery from exhaustive exercise implying the highest rate of release of muscle octopine into the bloodstream at this time. In support of this suggestion the data in Table 2 show that blood octopine concentrations are also highest under these conditions. Blood octopine levels were elevated during hypoxia, recovery from hypoxia, and recovery from exercise but not during exercise itself implying that there may be a restriction of blood flow to the muscle bed during strenuous exercise.

While the absolute increases observed in blood octopine concentration are quite small (particularly compared to the blood lactate levels measured after vertebrate muscle work), the percentage increases in blood octopine concentration after exercise or hypoxia stress are large. Blood octopine concentration at any given time is the net balance between the rate of octopine output into the blood versus the rate of octopine uptake from the blood. Significant amounts of muscle octopine could be moved in the blood without a large absolute increase in blood octopine levels if the rate of release from muscle and the rate of uptake by other tissues were similar. Given that the rate of octopine uptake by non-muscular tissues is governed by the rate of aerobic oxidation of the compound, a disruption of aerobic metabolism during the recovery period should result in a net increase in blood octopine. Indeed, when exercised animals were allowed to recover in hypoxic water (which would restrict the aerobic oxidation of octopine), blood octopine levels rose to 8-fold those of the controls. Thus it appears that a significant portion of the octopine produced in mantle muscle can be released into the bloodstream for oxidation by other tissues.

The oxidation of lactate produced in vertebrate skeletal muscle by other tissues of the body is necessitated by the low endogenous rates of lactate oxidation in muscle. A similar low capacity for octopine oxidation in mantle muscle is indicated by the ^{14}C-octopine studies. Mantle muscle showed the lowest relative capacity for the uptake of blood ^{14}C-A-octopine of any tissues tested. In addition, the oxidation of ^{14}C-A-octopine to release ^{14}C-arginine by resting muscle was found to be minimal compared to the six to eight fold higher rates of oxidation of ^{14}C-A-octopine by tissues such as brain and ventricle. The ^{14}C-P-octopine experiments indicated that of octopine oxidized by muscle, the pyruvate moiety is utilized as an aerobic substrate by the Krebs cycle. This is likely the only fate of the pyruvate derived from the reversal of the octopine dehydrogenase reaction in muscle as the activities of gluconeogenic enzymes in mantle muscle are extremely low (Hochachka et al., 1975; Storey, unpublished data). The endogenous oxidation of octopine in mantle muscle is likely restricted by the presence of an octopine dehydrogenase isozyme in this tissue which kinetically resembles the M form of lactate dehydrogenase (Storey and Storey, 1979). This isozyme has a high affinity for pyruvate and arginine but displays very high K_m's for octopine and NAD^+. Thus although the octopine dehydrogenase reaction is freely reversible, it appears that the kinetic properties of mantle muscle octopine dehydrogenase, coupled with the overall metabolism of the mantle muscle, do not gear the muscle for the effective use of octopine as an aerobic substrate.

However, octopine may be an efficient aerobic substrate for extramuscular tissues such as brain, heart, gill, and hepatopancreas. All of these tissues showed high rates of removal of ^{14}C-A-octopine from the bloodstream and all appeared able to efficiently use blood octopine as a substrate even at the very low blood octopine concentrations used in the ^{14}C-A-octopine experiments. The breakdown of ^{14}C-A-octopine was readily carried out by tissues such as brain, ventricle, and gill resulting in ^{14}C-arginine levels of up to 40% of the total tissue radioactivity 20 min after injection of ^{14}C-A-octopine. Octopine is clearly, therefore, an important aerobic substrate for a number of tissues.

Cephalopod brain is a highly aerobic tissue and appears, from the data for ^{14}C-glucose uptake, to be highly dependent upon blood glucose as its major substrate. However, the present studies also show that octopine can also be a major aerobic substrate for the brain when the compound is present in the bloodstream. During recovery from muscular exercise, the oxidation of blood octopine released from mantle muscle by the brain and other aerobic tissues could allow the diversion of blood glucose into the resynthesis of muscle glycogen reserves. The high capacity for octopine oxidation demonstrated by brain and by other cephalopod tissues may be largely dependent upon the presence of the brain specific isozyme of octopine dehydrogenase (Storey, 1977). This isozyme, which occurs mixed with the muscle form in a number of other soft tissues, has a number of kinetic properties which strongly resemble those displayed by the H isozyme of lactate dehydrogenase (Storey and Storey, 1979). Chief among these is a strong substrate inhibition by pyruvate, a property which could limit the effectiveness of the enzyme in octopine synthesis under conditions of high glycolytic flux. In the direction of octopine oxidation, however, the brain isozyme displays a very low K_m for octopine 0.09 mM), a value well within the range of in vivo blood octopine concentrations seen in this study. Thus the high titres of the brain isozyme of octopine dehydrogenase found in the brain appear to be geared not to the anaerobic production of octopine but to the efficient use of octopine as an aerobic substrate.

The release of muscle octopine into the bloodstream for oxidation in other tissues results in a depletion of the muscle arginine pool. A large scale drain of the arginine pool could inhibit the rate of restoration of arginine phosphate levels in the muscle during the recovery from exercise. But, as Table 1 demonstrates, even after exhaustive exercise, the free arginine reserves of the muscle are always sufficient to catalyze the complete restoration of muscle arginine phosphate stores. Thus the resynthesis of arginine phosphate reserves need not be obligately tied to the oxidation of muscle octopine. Blood arginine levels in *Sepia* are low (<0.03 μmol/ml; Storey, unpublished data) as are the arginine/arginine phosphate pools in most tissues other than mantle muscle. The present data indicate that ^{14}C-arginine is readily taken up from the blood by mantle muscle and it would appear therefore that the arginine moiety released during octopine oxidation in tissues such as brain and ventricle could be recycled via the bloodstream to reenter the muscle arginine pool.

The role of the hepatopancreas in octopine catabolism is not yet clearly understood. Hepatopancreas took up significant amounts of ^{14}C-A-octopine and in the case of ^{14}C-P-octopine, incorporation of the ^{14}C-pyruvate moiety into Krebs cycle carboxylic acids was detected. The organ could be important in the interconversion and storage of dietary substrates. Indeed, the rapid intercorporation of ^{14}C-glucose into glycogen and the presence of substantial glycogen reserves (approx. 20 μmol/g wet wt.) in the organ indicate a possible role in the overall carbohydrate metabolism of the animal. Based on preliminary data for gluconeogenic enzyme activities in the hepatopancreas, the organ could be a major site of gluconeogenesis in the cephalopod (Storey, unpublished data), and as such could be involved in the utilization of the pyruvate moiety of octopine as a gluconeogenic substrate. A modified Cori cycle could exist in the cephalopod. Octopine released from the muscle could be catabolized by the hepatopancreas, the pyruvate moiety being reconverted to glucose. Glucose and arginine would then be recycled to the muscle.

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