Octopine metabolism in *Sepia officinalis*: effect of hypoxia and metabolite loads on the blood levels of octopine and related compounds

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Male *Sepia officinalis* were subjected to hypoxia and the concentrations of blood metabolites were measured during stress and recovery. Blood octopine levels were elevated during hypoxia, whereas blood glucose concentration declined. During recovery, octopine was rapidly cleared from the blood while blood glucose concentration increased, initially overshooting the control level, before returning to prehypoxia levels.

The clearance of an octopine bolus (300 µmol given intravenously) from the blood was followed. Octopine uptake from the blood was correlated with a transient rise in blood glucose concentration. Injection of an arginine bolus resulted in an increase in blood octopine levels, whereas a lactate bolus led to elevated blood glucose and octopine levels.

The data show that octopine concentration in cephalopod blood is modulated in response to physiological stress and that octopine metabolism is closely integrated with the metabolism of glucose, arginine, and lactate. It is suggested that the octopine produced during glycolytic muscular work is transported via the bloodstream for use as an aerobic substrate in other tissues. The inverse relationship between blood octopine and glucose levels suggests the presence of a modified "Cori cycle" in which octopine released from muscle can be taken up by tissues capable of utilizing the compound as a gluconeogenic substrate.

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Des *Sepia officinalis* mâles ont été soumis à une hypoxie; la concentration des métabolites du sang a été mesurée durant le stress et pendant la récupération. La concentration d'octopine sanguine est élevée durant l'hypoxie, alors que la concentration de glucose diminue. Durant la période de récupération, l'octopine disparaît rapidement du sang, alors que la concentration de glucose augmente pour atteindre un niveau supérieur au niveau normal avant de retourner au point initial.

Une dose d'octopine (300 µmol) a été administrée par injection intraveineuse. La libération de l'octopine du sang est associée à une augmentation temporaire de la concentration de glucose. L'injection d'une dose d'arginine augmente la concentration d'octopine dans le sang et l'injection d'une dose de lactate augmente à la fois la concentration de glucose et la concentration d'octopine du sang.

Les données démontrent que la concentration d'octopine dans le sang, chez les céphalopodes, fluctue en réaction à un stress physiologique et que le métabolisme de l'octopine est fortement relié au métabolisme du glucose, de l'arginine et du lactate. Il se peut que l'octopine produite durant un travail musculaire glycolytique soit transportée par le sang et utilisée comme substrat aérobique dans les autres tissus. La relation inverse entre les concentrations d'octopine du sang et celles du glucose permet de croire à l'existence d'un "cycle de Cori" modifié où l'octopine libérée par les muscles serait récupérée par les tissus capables de s'en servir comme substrat gluconéogénique.

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Introduction

Octopine (octopine dehydrogenase reaction = pyruvate + arginine + NADH ⇌ octopine + NAD$^+$) is the end product of anaerobic glycolysis in the muscles of cephalopod molluscs (Storey and Storey 1979*a*; Hochachka *et al.* 1977; Grieshaber and Gade 1976). In *Sepia officinalis*, octopine production has been demonstrated in the mantle muscle during both hypoxic stress and muscular work (Storey and Storey 1979*a*). The octopine – octopine dehydrogenase system of cephalopods is in many ways analogous to the lactate – lactate dehydrogenase system of vertebrate tissues. Oc-

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topine is produced by the muscle in high concentrations during work, is found in elevated concentrations in the blood during recovery, and is readily taken up from the bloodstream and oxidized by a variety of other tissues including brain and ventricle. Tissue-specific isozymes of octopine dehydrogenase occur (Storey 1977; Storey and Storey 1979*b*). The muscle isozyme appears geared to the rapid synthesis of octopine during glycolytic activity, whereas the brain specific isozyme has properties closely resembling those of the H isozyme of lactate dehydrogenase and appears well suited for a function in octopine oxidation. Thus there is good evidence to suggest that muscle octopine, like the lactate produced during vertebrate muscle work, could be an effective substrate for aerobic catabolism or for gluconeogenesis in other body tissues.

In the present study we have examined the time course of changes in blood octopine concentration during hypoxia and recovery with the aims of establishing the pattern and extent of octopine release from the muscle. Experimental manipulations of blood octopine levels were also used to study the rate of octopine clearance from the blood and to determine the relationship between blood octopine concentration and the concentrations of other blood metabolites, most notably glucose. The experiments demonstrated that octopine concentration in the blood was elevated during hypoxia but octopine rapidly returned to control levels upon return to normoxic water. Experimental manipulation of blood octopine concentration resulted in a transient rise in blood glucose during the time of octopine clearance. Additionally, blood octopine levels were affected by the experimental elevation of the concentrations of blood arginine and lactate.

Materials and Methods

Chemicals and Animals

Biochemicals and coupling enzymes were purchased from Boehringer Mannheim Corp. Octopine and arginine kinase were from Sigma Chemical Co. In preparation for cannulation of the cephalic vena cava with an indwelling polyethylene catheter (PE80), male cuttlefish, *Sepia officinalis* (1600–2800 g), were immersed in ice-cold seawater. The resulting muscle relaxation gave access to the cephalic vena cava near the anus where the vessel runs superficially. The vessel was cannulated nonobstructively through a small hole made in the vessel wall percutaneously by a cutting-edge suture needle. The catheter was advanced upstream for 5–10 cm and was stabilized by anchoring sutures to the connective tissue alongside the vessel and the ventral wall of the siphon. The length of a catheter was about 80 cm and terminated in a light-weight stopcock through which venous blood could be sampled and (or) injections made without disturbing the animal. Following a cannulation an animal was allowed to recover for a minimum of 10 h before experimentation. The animals showed no adverse response to surgery and appeared healthy for at least 7 days.

Hypoxia Experiments

Individual animals were placed one at a time in a small tank and the P_{O_2} of the water lowered from 140 to 45 mmHg (1 mmHg = 133.322 Pa) by bubbling with N_2 gas. The nitrogen was then turned off and this point designated as the start of hypoxia. Hypoxia was continued for 45–75 min depending on the experiment, after which animals were removed to normoxic water for recovery. Water and blood P_{O_2} were determined using a Radiometer O_2 electrode (E 5045) and a Radiometer PHM 72 acid–base analyzer. Blood samples were taken at intervals during hypoxia and recovery and analyzed for metabolite levels as described below. Throughout hypoxia the seawater P_{O_2} continued to decline slowly ($P_{O_2}$was 19–31 mmHg at the end of hypoxia) and the animals, although remaining quiet throughout, showed signs of distress. During the most severe phase of hypoxic exposure, venous P_{O_2} showed values matching those of ambient water suggesting that no O_2 was taken up from the water or utilized by the tissues.

Metabolite Bolus Experiments

To experimentally raise the blood concentration of a metabolite, animals were injected via the chronically implanted catheter with one of three loads (each in 3 mL seawater): 300 μmol octopine, 300 μmol Na· lactate, or 600 μmol L-arginine. Infusion of the bolus was followed by 0.5 mL seawater to flush out the catheter. Animals were kept in normoxic water throughout and were undisturbed by injection. Blood samples were taken at timed intervals and the time course of clearance of a metabolite load from the blood followed.

Blood Sampling and Preparation of Extracts

To withdraw a blood sample, a 1-mL syringe was fitted to the stopcock and the stopcock was opened. Blood drawn from the "dead space" inside the catheter was discarded and a further 0.5-mL sample was withdrawn. Blood was ejected into 1 volume of ice-cold 8% perchloric acid in 40% ethanol. Precipitated protein was removed by centrifugation and the extract neutralized by addition of 3 *M* K_2CO_3 in 0.5 *M* triethanolamine, pH 6.0 (Williamson and Corkey 1969). After centrifugation, neutralized samples were stored at −20°C.

Metabolite Assays

Octopine

Octopine dehydrogenase (purified free of lactate dehydrogenase) from *Sepia* mantle muscle was prepared as described by Fields *et al.* (1976) with the addition of a gel filtration step using Sephadex G-200. Octopine in the blood was measured spectrophotometrically in an assay containing 100 m*M* Tris buffer, pH 9.0, 2.0 m*M* NAD^+, 10 m*M* EDTA, 100 μL neutralized blood sample, and 100 μL purified octopine dehydrogenase in a final volume of 1 mL. Addition of EDTA to the assay for the analysis of blood octopine concentration was found to be necessary (J. Fields, personal communication); this may be due to high concentrations of metal ions in the blood extract. Glucose and lactate were measured by the methods of Lowry and Passonneau (1972) and arginine was determined as described by Storey and Storey (1978).

Results

The effect of hypoxia on blood metabolite levels is shown in Fig. 1. Blood glucose levels declined steadily during hypoxia but upon return to normoxic water, glucose concentrations rapidly increased, overshooting the control level before gradually returning to the prehypoxia state. Blood

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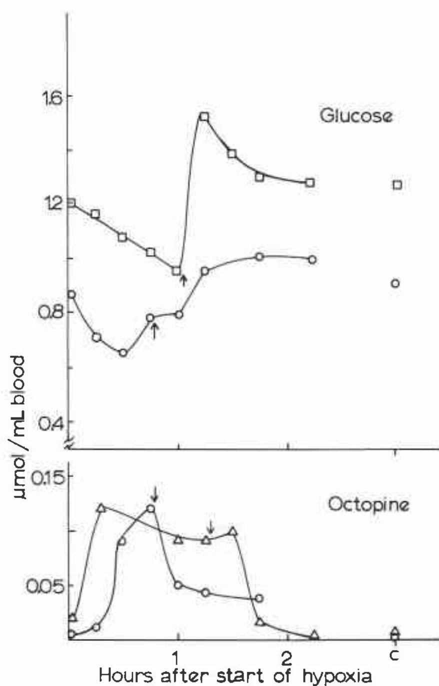

FIG. 1. Blood octopine and glucose concentrations in *Sepia officinalis* during hypoxia and recovery. The length of hypoxic excursion was timed from the point at which seawater P_{O_2} reached 45 mmHg. Animals were returned to normoxic water at the times indicated by the arrows. Blood samples were taken at intervals during hypoxia and recovery. Blood glucose and octopine levels are presented in the upper and lower portions of the figure, respectively. Control levels of these metabolites are shown at the right. Each curve is a summary of the data from an individual animal.

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octopine concentrations showed an inverse pattern. Normoxic control levels of octopine were very low (≤ 0.02 µmol/mL) in all animals tested. During hypoxia, blood octopine levels rose rapidly, remaining elevated until the return to normoxic water. At this point blood octopine was quickly cleared. Blood arginine levels remained low (≤0.03 µmol/mL) and constant throughout hypoxia and recovery as did blood lactate concentrations (0.10 µmol/mL).

In light of the rapid rate at which blood glucose and octopine concentrations changed in the previous experiments, we reasoned that useful data could be gained by administering a metabolite bolus injected intravenously and by following the clearance curve and related changes in the levels of other blood metabolites.

The clearance curves for octopine loads (300 µmol of octopine) are shown in Fig. 2. The octopine load was rapidly distributed in the blood pool, resulting in an initial (3 min postinjection) blood octopine concentration of 2.7–4.7 µmol/mL,

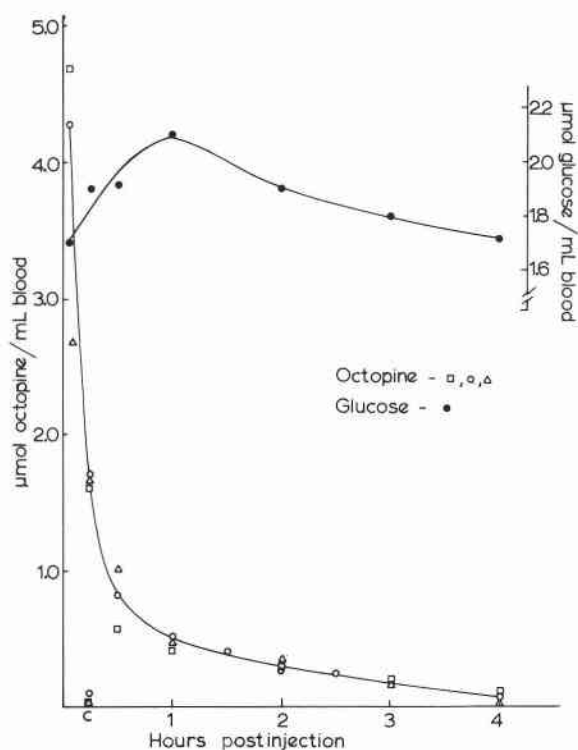

FIG. 2. Blood octopine and glucose levels in *Sepia officinalis* after intravenous injection of 300 µmol octopine. Control blood octopine levels are shown at the lower left. ○, △, □, octopine clearance curves for three individuals; ●, blood glucose levels.

dependent upon individual animal size. The curve showing octopine clearance from the blood appeared to be approximately hyperbolic, with half of the octopine being removed in 12 to 14 min (based on an initial 3-min sample). Blood octopine concentration returned to control levels after about 4 h. During octopine clearance, blood glucose levels were elevated, peak glucose concentration occurring approximately 1 h after injection. No significant changes in lactate or arginine concentrations in the blood were found during the clearance of the octopine load.

When lactate was administered in a bolus (300 µmol), the compound was also quickly cleared from the blood (lactate levels were reduced by half in 12 min), normal blood levels of the weak acid being reached after 3 h (Fig. 3). An immediate, but small, increase in blood octopine was seen during the clearance of the lactate load. Blood glucose levels were also elevated temporarily. No change in blood arginine concentration was found.

Arginine occurs in high concentrations intracellularly in *Sepia* mantle muscle (30–45 µmol/g wet weight (Storey and Storey 1979*a*)) but is almost

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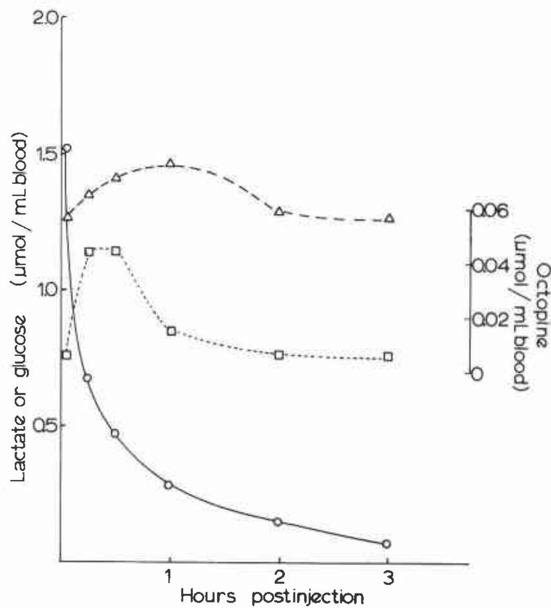

FIG. 3. Blood lactate, octopine, and glucose concentrations in *Sepia officinalis* after intravenous injection of 300 µmol lactate. ○, lactate clearance curve; △, blood glucose □, blood octopine.

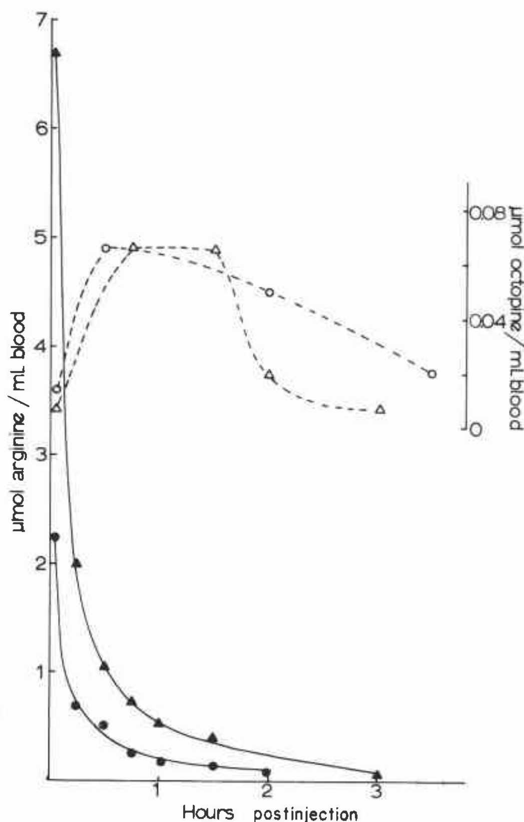

FIG. 4. Blood arginine and octopine levels in *Sepia officinalis* after intravenous injection of 600 µmol arginine. ●, ■, arginine clearance curves for two individuals; ○, △, blood octopine for two individuals.

undetectable in the blood. Both enzymatic and amino acid analysis of blood arginine content showed that control blood levels in *Sepia* were in the 0.01–0.03 µmol/mL range. The arginine bolus clearance curve (blood arginine levels were reduced by half in 9 min) is shown in Fig. 4. Arginine clearance was accompanied by a transient increase in blood octopine levels. Glucose and lactate levels in the blood were unaffected.

Discussion

In this study, the use of an indwelling catheter allowed the measurement of venous blood octopine concentrations (also glucose, lactate, and arginine levels) during the course of hypoxic stress and recovery in *Sepia*. These data, coupled with the analyses of the effects of octopine, arginine, and lactate boluses upon blood metabolite levels, add some new insights into the metabolism of the glycolytic end product, octopine, by cephalopod tissues.

During the hypoxic stress and subsequent recovery, an inverse relationship between blood glucose and octopine concentrations was found, implying that the two compounds may be closely linked metabolically. The steady decrease in blood glucose levels throughout the course of the hypoxic excursion is likely the result of an increased role for this blood sugar as a tissue substrate during hypoxia. The hypoxic state would impose an increased dependence upon anaerobic glycolysis for energy production in a number of tissues and the increased circulating levels of octopine seen during hypoxia are likely, therefore, a reflection of the increased tissue production of octopine as a glycolytic end product. Indeed, previous studies have shown that octopine concentration in mantle muscle of *Sepia* rises to approximately 4 µmol/g wet weight under similar conditions of hypoxic stress (Storey and Storey 1979*a*).

During the recovery from hypoxia, blood glucose levels rapidly increase and return to normal concentrations while blood octopine is rapidly cleared. The slight time delay in the initiation of octopine clearance from the blood may indicate a continued release of tissue octopine into the bloodstream for some time after the return to normoxic water. The metabolic fate of end-product octopine appears to be twofold. After reversal of the octopine dehydrogenase reaction, the arginine moiety can be reincorporated into tissue arginine pools, whereas the pyruvate moiety can be (1) utilized as a substrate of aerobic metabolism in the Krebs cycle or (2) incorporated into gluconeogenic reactions. The low rates of octopine oxidation by mantle muscle (Storey and Storey 1979*a*) and the lack of

significant activities of gluconeogenic enzymes (Hochachka *et al.* 1975) suggest that the catabolism of muscle octopine must take place largely in other tissues of the body, mediated by the transport of octopine in the blood.

The presence of a modified form of the Cori cycle involving octopine, arginine, and glucose cycling between tissues in cephalopods has been suggested (Storey and Storey 1979*a*). The present study provides further evidence for the existence of this cycle. The correlation between increasing blood glucose and decreasing blood octopine levels during recovery from hypoxia suggests that the restoration of blood glucose levels may be partially accomplished via reactions reconverting the pyruvate moiety of octopine into glucose. The effects of the octopine loads on blood glucose levels (Fig. 2) lend further support to this suggestion. Here tissue uptake and catabolism of high amounts of octopine, without the addition of the hypoxic stress, has a direct effect in increasing blood glucose concentration. Similarly, tissue handling of the lactate bolus resulted in a temporary increase in blood glucose (Fig. 3). Both octopine and lactate, by a reversal of the octopine dehydrogenase or lactate dehydrogenase reactions, provide pyruvate for gluconeogenesis and could therefore stimulate glucose production in certain tissues. Glucose is then released into the circulation for use as a substrate by tissues such as mantle muscle.

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The relationships between blood metabolites in *Sepia* were further investigated in experiments in which lactate or arginine loads were administered. A strong tissue uptake of these compounds was demonstrated by their rapid clearance from the blood and both compounds, presumably through their effects on tissue metabolism, were found to influence the blood levels of octopine. Both arginine and lactate (after conversion to pyruvate) provide substrate for the octopine dehydrogenase reaction. Control of this reaction in muscle appears to be through the interacting effects of substrate concentrations, increasing concentrations of one substrate (pyruvate or arginine) decreasing the K_m of the enzyme for the other substrate (Fields *et al.*, 1976). The presence of increased intracellular levels of pyruvate or arginine due to the uptake of the metabolite boluses could have an effect, therefore, in temporarily increasing octopine production in some tissues with a resultant increase in blood octopine levels.

The rapid clearance of the lactate bolus and its effect in raising blood glucose levels implies a reasonable capacity for the catabolism of lactate in *Sepia* tissues. In light of the extremely low activities of lactate dehydrogenase in mantle muscle (1.6 units/g wet weight vs. 100 units for octopine dehydrogenase (Storey 1977)), these results might not have been expected. But, although lactate is probably of little significance as an end product in mantle muscle, the compound may play a much greater role in the metabolism of other tissues. Ventricle, branchial heart, and brain all have significant activities of lactate dehydrogenase, and in gill and stellate ganglion, lactate dehydrogenase activity exceeds that of octopine dehydrogenase (Storey 1977). Indeed, glycolysis in many of the soft tissues of *Sepia* may lead preferentially to lactate production, whereas the presence of octopine dehydrogenase in these tissues may be to allow the catabolism of muscle-produced octopine as an aerobic substrate.

Although a significant increase in blood octopine concentration during stress has been demonstrated by this and a previous study (Storey and Storey 1979*a*), the absolute concentration of octopine in *Sepia* blood is never high (<0.2 μmol/mL), especially when contrasted with muscle octopine levels, which can reach 13 μmol/mL after exhaustive swimming. The significance of octopine transfer between the octopine-producing mantle muscle and tissues capable of utilizing octopine as an aerobic substrate could, therefore, be doubted. A major result of the metabolite bolus experiments, however, is the clear demonstration of the very rapid and efficient manner in which metabolites are taken up from the blood by *Sepia* tissues. Clearance of amounts of octopine and arginine up to 200 times the normal blood concentrations was largely completed within a few minutes, which indicates a strong control over blood metabolite concentrations in *Sepia*. In a previous study it was demonstrated that the tissues of *Sepia* could rapidly concentrate (and oxidize) tracer amounts of [^{14}C]octopine administered intravenously (Storey and Storey 1979*a*). These data, when considered along with the very rapid circulation times recorded for cephalopods, would indicate, therefore, that a significant exchange of metabolites between tissues could take place without large changes in the measured blood metabolite concentrations.

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