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EVIDENCE FOR A ROLE OF GABA IN METABOLIC DEPRESSION DURING ANOXIA IN CRUCIAN CARP (*CARASSIUS CARASSIUS*)

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Summary

Anoxia-tolerant vertebrates decrease their metabolic rate by 70 % or more during anoxia, while the major inhibitory neurotransmitter in the vertebrate brain, GABA, increases in concentration. To test the possibility that GABA could be a mediator of anoxic metabolic depression, crucian carp (*Carassius carassius* L.) were given an inhibitor of GABA synthesis, isoniazid, and the rate of anoxic ethanol production (ethanol being the main end product of energy metabolism in anoxic crucian carp) as well as the rates of normoxic and hypoxic oxygen consumption were measured. Isoniazid (500 mg kg^{-1}), which significantly inhibited the anoxia-induced rise in brain GABA concentration, caused a nearly threefold increase in anaerobic ethanol production without affecting normoxic oxygen consumption. The GABA synthesis inhibitor 3-mercaptopropionic acid (3-MP, 200 mg kg^{-1}) and the GABA receptor antagonist securinine (20 mg kg^{-1}) caused similar rises in anoxic ethanol production. Nevertheless, crucian carp given 250–500 mg kg^{-1} isoniazid, 200 mg kg^{-1} 3-MP or 20 mg kg^{-1} securinine all recovered after anoxia, suggesting that a complete depression of the rate of metabolism may not be essential for survival during short-term anoxia. These results indicate that GABA is a mediator of anoxic metabolic depression in crucian carp.

Introduction

Only a small number of vertebrates can survive more than a few minutes of anoxia, with the brain showing particular susceptibility to anoxic damage. The main reason for this low anoxia tolerance is probably the high energy demands of the brain coupled with an inability to depress energy consumption during anoxia. In the anoxic mammalian brain, anaerobic energy production is unable to match energy consumption. During anoxia, there is a rapid fall in the ATP levels, leading to a decreased rate of Na^+/K^+ pumping and a net outward leakage of K^+ from the neurons. This is soon followed by a more or less general depolarization,

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presumably caused by the opening of voltage-gated Na^+, Ca^{2+} and K^+ channels as well as by a release of excitatory amino acids, notably glutamate (reviewed by Siesjö, 1990). Indeed, it is becoming increasingly clear that neurotransmitters play important roles in the development of anoxic brain damage.

Freshwater turtles, crucian carp (*Carassius carassius* L.) and goldfish (*Carassius auratus* L.) are among the few vertebrates that readily survive anoxia for many hours or even days at room temperature (Ultsch, 1989). These animals have been found to maintain relatively constant brain ATP levels during anoxia (Lutz *et al.* 1984; Kelly and Storey, 1988; Van der Boon, 1990), and it is assumed that, by maintaining their ATP levels, anoxia-tolerant vertebrates prevent the disastrous events that occur in the anoxic mammalian brain.

Metabolic depression is regarded as a major strategy for anoxic survival in anoxia-tolerant animals in general (Hochachka, 1986; Hochachka and Guppy, 1987; Rosenthal *et al.* 1988; Storey, 1988) and is thought to be a key mechanism in maintaining brain ATP levels during anoxia in anoxia-tolerant vertebrates (Lutz *et al.* 1986; Rosenthal *et al.* 1988; Chih *et al.* 1989). Freshwater turtles have been found to undergo a marked metabolic depression during anoxia, and, in anoxic goldfish, systemic energy consumption is depressed by 70 % (Van Waversveld *et al.* 1989).

With regard to the depression of energy *production* during anoxia, covalent modifications of regulatory enzymes involved in glycolysis, as well as lowered levels of the glycolytic activator fructose 2,6-bisphosphate and increased levels of the glycolytic inhibitor alanine, are thought to mediate a decreased rate of glycolysis (Storey, 1987, 1988; Rahman and Storey, 1988; Nilsson *et al.* 1990). However, a key question must be how the rate of energy *consumption* is decreased, since a unilateral decrease in energy production would be disastrous, leading to a fall in ATP concentration followed by a loss of ion homeostasis and general depolarization (as in the anoxic mammalian brain). Thus, the decrease in energy production seen in anoxia-tolerant animals must be tightly linked with a decrease in energy consumption. The mechanisms responsible for the depression of energy consumption are largely unknown. Moreover, since few successful attempts have been made to inhibit metabolic depression, there has been a lack of experimental evidence to show that a decrease in energy consumption in anoxia-tolerant vertebrates really is essential for avoiding an initial disastrous fall in ATP levels (short-term survival). Metabolic depression could merely be a way of saving fuel, thereby greatly extending the survival time in anoxia (long-term survival). I recently found that crucian carp can survive at least a few hours of anoxia in spite of a marked inhibition of systemic metabolic depression caused by an adenosine receptor antagonist (Nilsson, 1991).

Principal representatives of anoxia-tolerant vertebrates, crucian carp, freshwater turtles and sea turtles, have recently been found to respond to anoxia by increasing their brain levels of inhibitory amino acids such as GABA, glycine and taurine, while simultaneously decreasing the levels of the excitatory amino acid neurotransmitters glutamate and aspartate (Nilsson, 1990; Nilsson *et al.* 1990,

1991). At least in turtles, increased levels of inhibitory amino acids are also seen extracellularly, where they can interact with receptors (Nilsson and Lutz, 1991). Clearly, the possibility that increased levels of inhibitory amino acids mediate metabolic depression deserves further experimental examination.

GABA is the major inhibitory neurotransmitter in vertebrates, and the aim of the present study was to determine how an inhibition of GABAergic transmission affects the rate of systemic energy metabolism in anoxic and normoxic crucian carp. Metabolic rate during anoxia was measured as the rate of ethanol production. *C. carassius* and *C. auratus* have the unique ability, among vertebrates, to produce and excrete ethanol as the principal metabolic end product during anoxia (Johnston and Bernard, 1983). Thus, the rate of anoxic ethanol excretion can be used as a continuous index of anoxic metabolic rate in crucian carp (Nilsson, 1991).

Materials and methods

All acclimations and experiments were carried out in Uppsala tap water thermostatted at 18±1 °C.

Animals

Crucian carp, weighing 41±11 g (mean±S.D.), were caught in a pond near Uppsala, Sweden, in August. They were kept indoors in 500-l tanks (100 fish in each) continuously supplied with aerated water (18 °C). The fish were fed daily with commercial trout food (Ewos, Sweden). The artificial light automatically followed Hamburg's latitude and longitude. The experiments were carried out between September and December after at least 1 month of acclimation to the indoor conditions.

Drugs

Isonicotinic acid hydrazide (isoniazid), 3-mercaptopropionic acid (3-MP) and securinine were obtained from Sigma Chemical Co, St Louis, MO. Isoniazid and 3-MP were dissolved in 0.9 % NaCl. The isoniazid solution had a neutral pH. The pH of the 3-MP solution was made neutral by the addition of NaOH. 10 mg of securinine was first dissolved in 1 ml of 0.02 mol l^{-1} HCl, and then 0.5 ml of 0.1 mol l^{-1} Na_2HPO_4 was added to give a final pH of 6.0. All drugs were given intraperitoneally in a volume of 100–200 μl per 40 g of fish, and controls were given a corresponding volume of 0.9 % NaCl.

Measurements of ethanol production in anoxia

All measurements of ethanol production were carried out in a 24-l plastic (PVC) box that had been divided into 12 2-l chambers, with the sides and bottom of each chamber made of black PVC. Each chamber was equipped with an air stone for aeration or nitrogen bubbling, a small outlet with a valve for water sampling, and two 15 mm holes connecting it with neighboring chambers to allow water to flow

through the box during acclimation. The 24-l box was covered with a pyramid-shaped acrylic lid with a small pipe (gas outlet) in the center in order to avoid the formation of air pockets during nitrogen bubbling. The lid was covered with a semitransparent black plastic bag allowing dim light to penetrate. The fish (one in each chamber) were put into the box 24 h before the experiment. During this acclimation period, the chambers were aerated and supplied with continuously exchanged water. The drugs (or vehicles) were injected 60 min (isoniazid) or 15 min (3-MP and securinine) before the experiments. At the same time, the inlet and outlet for water to each chamber were plugged. The experiment was started by bubbling the water in each chamber with nitrogen, causing the O_2 level to fall below $0.1\,mg\,l^{-1}$ within 5–10 min. Water samples were taken regularly and the nitrogen bubbling continued during the whole experiment, causing the pH of the water to increase from 8.1 to 8.6 during a 5-h experiment. The experiments were carried out between 10:00 h and 17:00 h. For practical reasons, the time in anoxia was counted from the start of nitrogen bubbling. The ethanol concentration in 2 ml water samples was measured enzymatically using alcohol dehydrogenase (Krebs *et al.* 1969).

After the experiments with isoniazid, each fish was decapitated and the brain removed and frozen in liquid nitrogen within 1 min. GABA, glutamate and glutamine were measured after *o*-phthaldialdehyde derivatization using HPLC with fluorimetric detection, as described in detail by Nilsson (1990).

Measurement of O_2 consumption by closed respirometry

Isoniazid (or vehicle) was injected 90 min prior to the experiments. Each fish was put in a 2-l glass jar that, as in the experiments with ethanol production, was kept shaded but not completely dark. The tip of an O_2 electrode (Oximeter OXI 96 from Wissenschaftlich Technische Werkstätten in Weilheim, Germany) was inserted through the lid of the jar. To make water circulate over the electrode membrane, the electrode was equipped with a small magnetic propeller (supplied by the manufacturer) driven by a magnetic stirrer placed outside the jar. The small propeller caused only a small local water current and did not produce any water movements strong enough to force the fish to swim. The decline in O_2 concentration in the water was continuously recorded and the slope of this curve at different O_2 concentrations was used to calculate the rate of O_2 consumption by the fish.

Statistics

The results are presented as means±S.E.M. Statistical significance was tested using the Wilcoxon rank sum test.

Results

Effects of isoniazid on ethanol production and the recovery after anoxia

Isoniazid (250 and 500 $mg\,kg^{-1}$) significantly increased the amount of ethanol

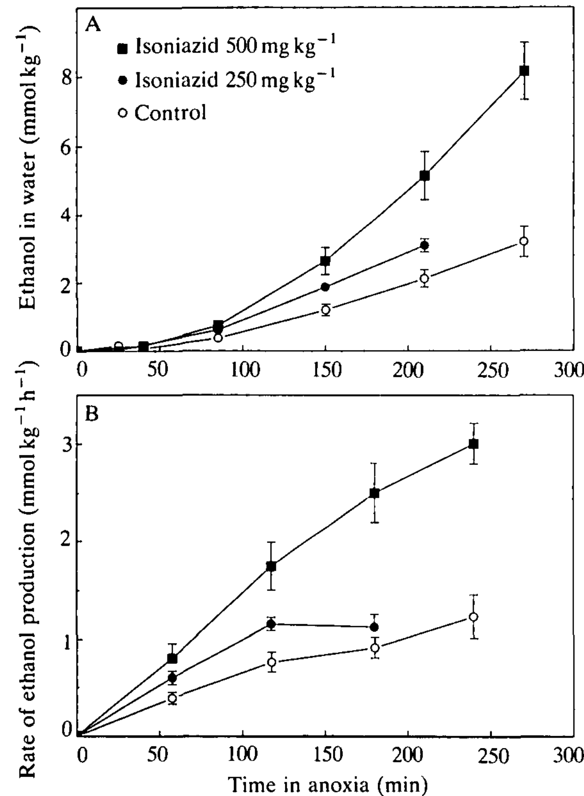

Fig. 1. (A, B) Effect of isoniazid on ethanol production in anoxic crucian carp. (A) The increase in the water ethanol concentration, per kilogram of fish, during 210 or 270 min of anoxia in controls (○, N=12), fish given 250 mg kg^{-1} isoniazid (●, N=6) and fish given 500 mg kg^{-1} isoniazid (■, N=6). (B) The rate of ethanol production at different times during anoxia calculated from the slope of the curves in A. Values are mean±S.E.

produced (Fig. 1A) and the rate of ethanol production (Fig. 1B) in anoxic crucian carp. At the higher dose, isoniazid caused the rate of ethanol production to increase to 2.1, 2.3, 2.7 and 2.4 times the control level after 60, 120, 180 and 240 min of anoxia (P<0.01 at each time), respectively (Fig. 1B). At the lower dose, a 1.5-fold increase was seen after 60 and 120 min (P<0.05 at each time), but the results suggested that the rate of ethanol production had returned to the control value after 180 min (Fig. 1B). Fish given 500 mg kg^{-1} isoniazid appeared comatose at the end of the exposure to anoxia, lying motionless on their side at the water surface.

Crucian carp given 500 mg kg^{-1} isoniazid and kept under normoxic conditions did not produce any measurable ethanol.

The effect of 1000 mg kg^{-1} isoniazid on anoxic ethanol production was also tested (data not shown). This dose of isoniazid caused 3.3- and 3.5-fold increases

in the rate of ethanol production after 210 and 270 min, respectively, of anoxia ($P<0.01$). However, unlike the lower doses, 1000 mg kg^{-1} isoniazid induced convulsions during anoxia and no more experiments were performed with such a high dose.

To test the effect of isoniazid on anoxic survival, crucian carp treated with 250 and 500 mg kg^{-1} isoniazid (1 h prior to anoxia) were exposed to 5 h of anoxia and then put into normoxic water. After anoxia, fish given 500 mg kg^{-1} isoniazid (N=4) displayed severe problems with their equilibrium (i.e. ability to keep upright). However, these problems were only temporary, lasting for about 40 h, and the fish were feeding again after 48 h. Crucian carp given 250 mg kg^{-1} isoniazid (N=4) did not show any apparent behavioral deficits after 5 h in anoxia. All these fish were alive and well more than 1 month after the experiment.

Effect of isoniazid on the brain levels of GABA, glutamate and glutamine

Fig. 2 shows the concentrations of GABA, glutamate and glutamine found in the brain of control and isoniazid-treated fish (250 and 500 mg kg^{-1}) after up to 5 h of anoxia. These are the same fish as those studied in the experiment presented in Fig. 1. Anoxic controls differed significantly from normoxic controls in all instances ($P<0.01$). Isoniazid, at a dose of 500 mg kg^{-1}, lowered the anoxia-induced increase in GABA concentration by 63 % ($P<0.001$). Thus, while the controls exposed to 270 min of anoxia had a brain concentration of GABA that was 106 $\mu g\ g^{-1}$ higher than that of normoxic fish, the increase in brain GABA concentration of fish given 500 mg kg^{-1} isoniazid was only 39 $\mu g\ g^{-1}$. Also, the anoxia-induced decreases in the brain levels of glutamate and glutamine (the main precursor of neuronal glutamate) were significantly ($P<0.05$) attenuated in fish treated with 500 mg kg^{-1} isoniazid. This is the expected result, since isoniazid inhibits the formation of GABA from glutamate. However, with the lower dose of isoniazid (250 mg kg^{-1}) no significant effects remained after 210 min of anoxia, although there was a tendency towards a lowered GABA level. This is in agreement with the results obtained from the measurements of ethanol levels in that, after 210 min of anoxia, a 250 mg kg^{-1} dose of isoniazid no longer caused any significant increase in the rate of ethanol production (Fig. 1).

Effects of isoniazid on oxygen consumption

The effect of isoniazid (500 mg kg^{-1}) on the routine metabolic rate in normoxia and hypoxia was studied using closed respirometry (Table 1; Fig. 3). In contrast to the drastic effect of isoniazid treatment on ethanol production during anoxia, isoniazid did not cause any change in the rate of oxygen consumption at normoxic oxygen tensions (6–8 mg $O_2\ l^{-1}$) (Table 1) when measured 120–180 min after injection.

Fig. 3 shows experiments where the fish were allowed to consume all the oxygen in the respirometer. Biphasic plots were obtained, very similar to those found with closed respirometry in common carp (Lomholt and Johansen, 1979). When meeting a declining ambient oxygen tension, the fish apparently decrease their

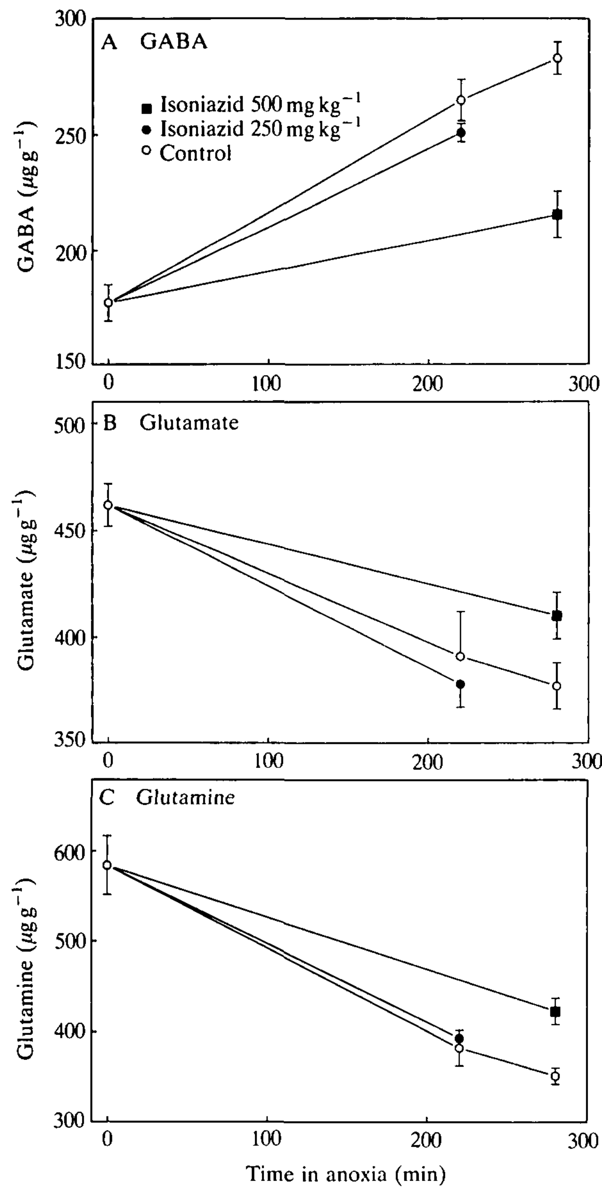

Fig. 2. (A–C) Effect of anoxia and isoniazid on the concentrations of GABA (A), glutamate (B) and glutamine (C) in crucian carp brain. The fish are those studied in the experiment presented in Fig. 1. The numbers of specimens are 6 (isoniazid) or 12 (control). Values are mean±S.E.

rate of oxygen consumption, possibly by lowering their physical activity and, at a certain oxygen tension, called the critical oxygen tension, they will be unable to take up all the oxygen needed to maintain a fully aerobic metabolism. Below the

Table 1. *Rates of normoxic oxygen consumption in isoniazid-treated (500 mg kg^{-1}) and control crucian carp*

	Rate of oxygen consumption (mg kg^{-1} h^{-1})	
Treatment	at 6.0–7.0 mg O_2 l^{-1}	at 7.0–8.0 mg O_2 l^{-1}
Control	156±17 (10)	168±12 (10)
Isoniazid	154±16 (6)	158±16 (8)

Values are means±S.E.M.
The number of specimens is given within parentheses.
No significant differences were found.

critical oxygen tension, the fish have to rely progressively on anaerobic metabolism. In these experiments, the slopes of the curves above the critical oxygen tension were significantly lower in fish given 500 mg kg^{-1} isoniazid (slope=9.9±3.0) than in controls (slope=18.1±2.0, P=0.032, Fig. 3), suggesting that isoniazid attenuates the ability of crucian carp to lower its oxygen consumption in response to a falling ambient oxygen tension. In contrast, isoniazid had no significant effect on the critical oxygen tension (1.4±0.2 mg l^{-1} in controls and 1.7±0.4 mg l^{-1} in isoniazid-treated fish).

The crucian carp is a tranquil species that is readily tamed, and the fish used were accustomed to daily handling. Nevertheless, since the measurements started only 30 min after the fish had been put in the respirometer, it is possible that the initial part of the oxygen consumption curves was influenced by stress.

Effects of 3-MP and securinine on ethanol production and the recovery after anoxia

The experiments with isoniazid suggested that inhibition of GABAergic neurotransmission increases the rate of anaerobic metabolism measured as ethanol production. To substantiate this suggestion, experiments were conducted with two other drugs known to inhibit GABAergic transmission in mammals.

Like isoniazid, both 3-MP and securinine drastically increased the rate of ethanol production in anoxic crucian carp (Figs 4, 5). 3-MP (200 mg kg^{-1} given 15 min prior to anoxia) caused the rate of ethanol production (Fig. 4B) to increase to 1.5, 2.3, 2.4, 2.0 and 1.4 times the control level after 70, 130, 190, 250 and 310 min, respectively, of anoxia (P<0.05 at 70 and 310 min; P<0.001 at 130–250 min). Thus, after 190 min, the effect of 3-MP started to decline. Securinine (20 mg kg^{-1} given 15 min prior to anoxia) was very fast-acting and potent in elevating the rate of ethanol production in anoxic crucian carp (Fig. 5B), causing 5.5-, 3.5-, 2.9- and 1.9-fold increases over control values after 20, 70, 135 and 195 min, respectively, of anoxia (P<0.01 at each time). The effect of securinine also declined with time, and the rate of ethanol production by securinine-treated fish was not significantly different from that of controls after 250 min of anoxia. In contrast to isoniazid (250 and 500 mg kg^{-1}) and 3-MP, securinine

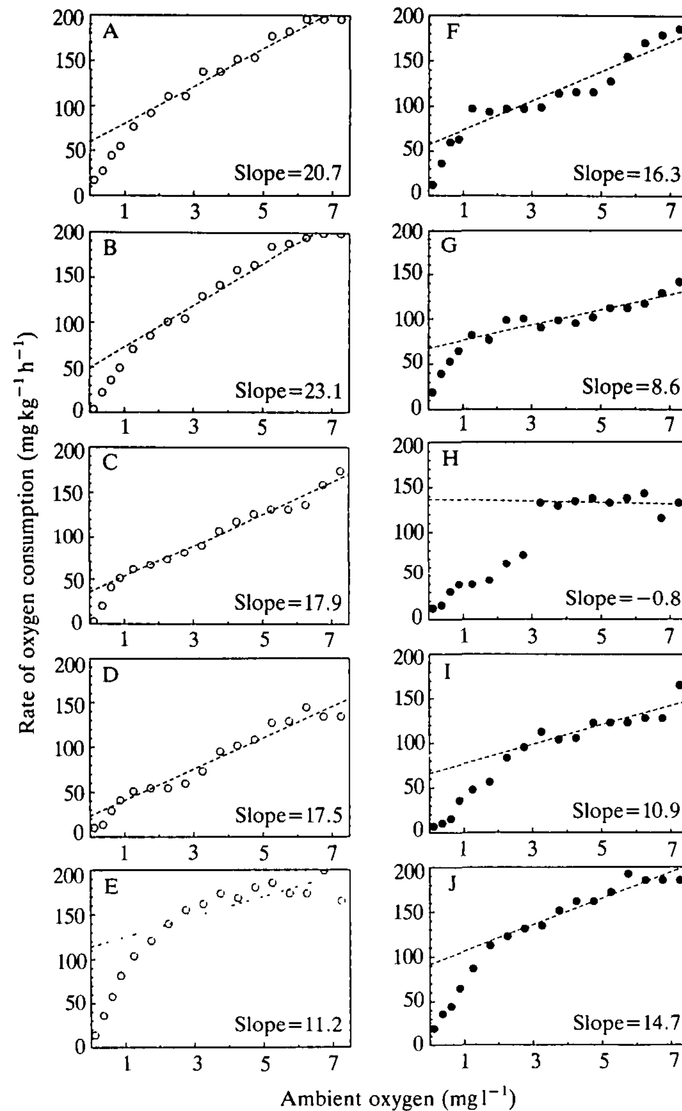

Fig. 3. (A–J) Effect of isoniazid on oxygen consumption in crucian carp studied by closed respirometry. (A–E) Control fish (○, given 0.9 % NaCl); (F–J) fish treated with isoniazid (●, 500 mg kg^{-1}). The decline in the water O_2 concentration in the respirometer was continuously recorded as the fish consumed the O_2. The slope of this curve at different O_2 concentrations was used to calculate the rate of O_2 consumption. It took between 4 and 6 h for the fish to consume all the O_2 in the respirometer. The regression lines are calculated from the values above the critical oxygen tension (i.e. above the inflection point). Each plot represents a different fish.

caused convulsions. However, the convulsions, which appeared only 10 min after injection, lasted less than 2 min. No convulsions were seen when the N_2-bubbling started.

After the exposure to anoxia, the crucian carp given 3-MP (200 mg kg^{-1}) or

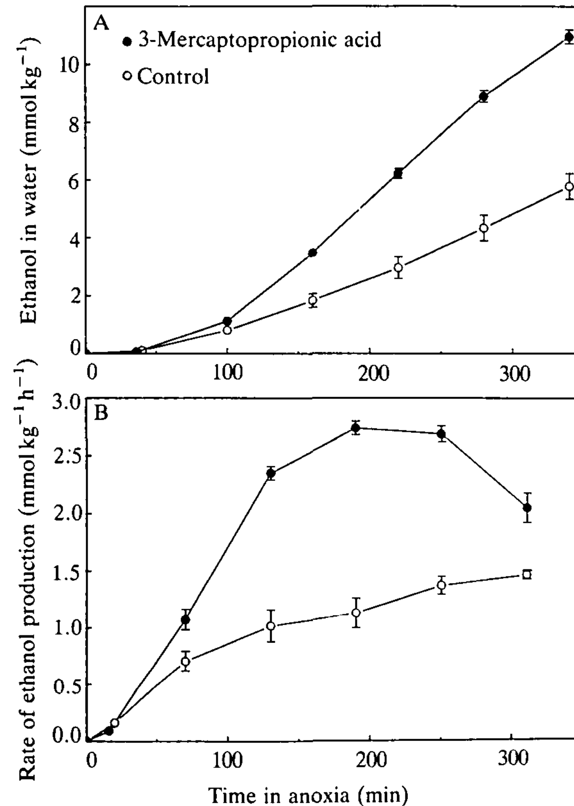

Fig. 4. (A, B) Effect of 3-mercaptopropionic acid (3-MP) on ethanol production in anoxic crucian carp. (A) The increase in water ethanol concentration, per kilogram of fish, during 340 min of anoxia in controls (○, N=6) and fish given 200 mg kg^{-1} 3-MP (●, N=6). (B) The rate of ethanol production at different times during anoxia, calculated from the slopes of the curves in A. Values are mean±S.E.

securinine (20 mg kg^{-1}) had lost their equilibrium and appeared more or less comatose. Nevertheless, these fish recovered after a few minutes of normoxia. The securinine-treated fish were alive and well for more than 2 months after the experiment, while fish given 3-MP were killed after 4 days in normoxia because edema fluid started to accumulate in their peritoneal cavity (probably as a result of infection or side effects of 3-MP at the injection site).

Discussion

Isoniazid, 3-MP and securinine, three drugs known to inhibit GABAergic transmission, were found to be highly effective in increasing the rate of anoxic ethanol production in crucian carp. It should be pointed out that the present results demonstrate that ethanol production in crucian carp can be altered pharmacologically, providing new possibilities for an insight into the mechanisms

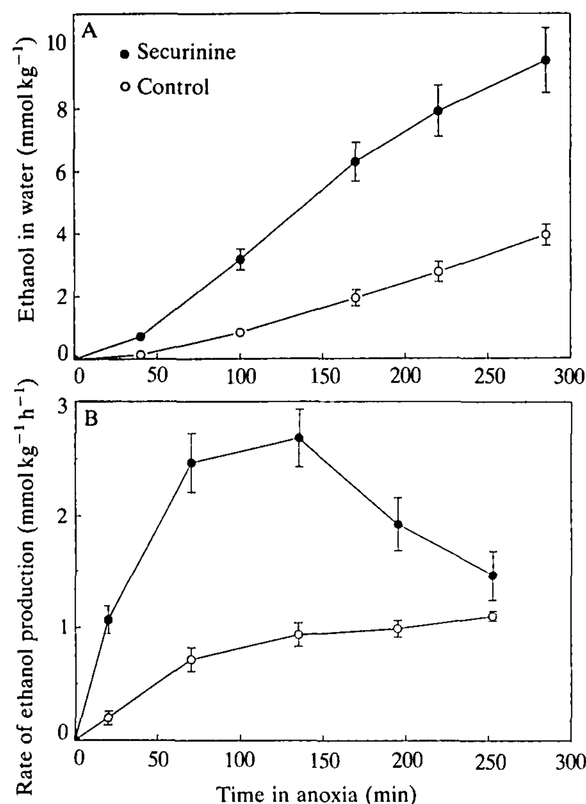

Fig. 5. (A, B) Effect of securinine on ethanol production in anoxic crucian carp. (A) The increase in water ethanol concentration, per kilogram of fish, during 280 min of anoxia in controls (○, N=6) and fish given 20 mg kg^{-1} securinine (●, N=4). (B) The rate of ethanol production at different times during anoxia, calculated from the slopes of the curves in A. Values are mean±S.E.

behind metabolic depression. Furthermore, since GABA is largely confined to the central nervous system, and since the brain of a 40 g crucian carp only constitutes 0.25 % of the body mass, the results indicate a key role for the brain in the regulation of systemic energy metabolism in crucian carp.

Ethanol is the major anaerobic end product in *C. carassius* and *C. auratus*, and the rate of ethanol production is probably a good measure of metabolic rate. Based on theoretical calculations, it has been suggested that *C. auratus* also produces some fat during anoxia (Van Waversveld *et al.* 1989), but the amount of fat produced would be too small to be measured experimentally. That a substantial increase in the rate of ethanol release to the water would reflect anything other than increased metabolic ethanol formation is unlikely. Ethanol readily penetrates biological membranes and is almost certainly excreted by passive diffusion alone. It has been shown that, after 6 h of anoxia, only 15 % of the total amount of ethanol produced by the crucian carp remains inside the body, the remainder

having been excreted (Johnston and Bernard, 1983). Thus, ethanol production in the two *Carassius* species provides a unique possibility to obtain a continuous measure of the metabolic rate in freely moving anoxic fish given different pharmacological treatments. Direct calorimetry was not a practical alternative in the present study. Apart from being laborious and requiring expensive equipment, the long equilibration time (hours) needed in direct calorimetry makes pharmacological manipulations impossible.

In *C. auratus*, anoxia causes a 70 % fall in heat production (Van Waversveld *et al.* 1989). In other words, metabolic depression produces a 3.3-fold difference between the metabolic rate in normoxia and that in anoxia. Consequently, the 2.7-fold increase in ethanol production found after injection of $500\,mg\,kg^{-1}$ isoniazid in *C. carassius* indicates a profound inhibition of metabolic depression, assuming that there is a relatively linear relationship between heat and ethanol production.

Moreover, isoniazid did not increase the routine metabolic rate during normoxia (Table 1), showing that the effect of isoniazid on ethanol production was not merely a reflection of an overall increase in metabolic rate due, for example, to behavioural arousal. Indeed, as pointed out in the Results, crucian carp given $500\,mg\,kg^{-1}$ isoniazid were more or less comatose after 270 min in anoxia, performing no physical activity while still having a highly elevated anoxic metabolic rate.

When the oxygen concentration in the closed respirometer reached hypoxic levels (Fig. 3), crucian carp treated with isoniazid seemed to be less able to depress their rate of oxygen consumption. As with the increase in anoxic ethanol production, this result indicates an inhibition of the ability to depress metabolic rate in response to a low ambient oxygen tension. Thus, the GABAergic system seems to be important for both the transition to and the maintenance of a depressed metabolic state.

To strengthen the suggestion that the increased rate of ethanol production in isoniazid-treated fish was caused by inhibition of GABAergic neurotransmission, two other anti-GABAergic drugs (3-MP and securinine) were tested for their effects on anoxic ethanol production. These drugs were also found to be highly effective in increasing anoxic ethanol production, although isoniazid, 3-MP and securinine all differ in the way in which they depress GABAergic neurotransmission. Isoniazid inhibits glutamate decarboxylase (GAD), the enzyme synthesizing GABA from glutamate, by inhibiting the binding of the cofactor pyridoxal phosphate or by decreasing pyridoxal phosphate concentrations (Horton *et al.* 1979). 3-MP also inhibits GAD, but by a different mechanism that does not involve pyridoxal phosphate (Lamar, 1970; Lindgren, 1983). In addition, 3-MP inhibits neuronal release of GABA (Fan *et al.* 1981), and extracellular brain levels of GABA have been found to fall drastically within 10 min of intraperitoneal 3-MP injection in rats (Kehr and Ungerstedt, 1988). Finally, the alkaloid securinine is a potent and selective $GABA_A$ receptor antagonist (Beutler *et al.* 1985; Farrant and Webster, 1989).

When interpreting the results obtained with these drugs, it should be kept in

mind that our knowledge of their effects on GABAergic neurotransmission comes from mammalian studies, and it is possible that they have different effects on fish. However, with regard to isoniazid, Fig. 2 clearly suggests that this drug act on fish by inhibiting GAD. Another possibility, which cannot be totally ignored, is that these drugs may stimulate hepatic ATP turnover and, thereby, anoxic energy consumption. Because of their chemical diversity, however, it seems unlikely that all three substances would have similar effects on liver metabolism. Moreover, such an effect would probably also be revealed by an increase in normoxic oxygen consumption.

The present results indicate that full metabolic depression may not be essential for survival during short-term anoxia in crucian carp. Treatment with isoniazid, 3-MP and securinine, causing a 2- to 5.5-fold increase in the anoxic metabolic rate lasting 3–4 h, apparently caused no permanent CNS damage. Thus, although 3-MP- and securinine-treated fish had lost their equilibrium at the end of the anoxic period, they recovered within a few minutes in normoxic water. However, the comatose state of these fish during anoxia indicates that a short-term inhibition of metabolic depression causes at least temporary CNS problems and, after isoniazid, the equilibrium problems lasted for about 40 h in normoxia.

GABA is apparently the major inhibitory neurotransmitter in the vertebrate brain. It is almost exclusively confined to the brain (Cooper *et al.* 1986), although some GABA is found peripherally (see below). This means that anti-GABAergic drugs are likely to have their most profound effects on the energy consumption of the brain. It is quite possible that the drugs used in the present study increased energy consumption of the brain to a greater extent than is indicated by the elevated rates of ethanol production, since ethanol production is a measure of systemic energy metabolism. Unfortunately, there is no existing method for measuring brain energy consumption *in vivo* in fish.

The brain of a crucian carp constitutes only 0.25 % of the body mass, so a depression of brain energy consumption *per se* would not have much effect on systemic energy metabolism. Thus, it is evident that energy utilization in other organs is also reduced during anoxia, and it is likely that such a shut down is controlled by the brain, acting through the peripheral nervous system and/or hormones. Target organs for metabolic depression could include intestine, kidney and liver, all of which have high specific metabolic rates in carp (Itazawa and Oikawa, 1986). Indeed, in the cod (*Gadus morhua*), hypoxia induces an increase in total systemic circulatory resistance and a profound decrease in the blood flow to the gastrointestinal region, which normally receives 40 % of cardiac output (Axelsson and Fritsche, 1991). Such circulatory changes appear to be mediated by adrenergic nerves as well as by circulating catecholamines (Fritsche and Nilsson, 1990; Axelsson and Fritsche, 1991). There is evidence for the presence of GABAergic neurons or terminals in the gastrointestinal tract (Jessen *et al.* 1987) and in the heart (Charbonneau *et al.* 1986) of mammals. Thus, it is possible that peripheral GABAergic systems are also involved in metabolic depression and that an inhibition of these systems may contribute to the increased rates of ethanol

production presently observed in anoxic crucian carp after anti-GABAergic drug treatment.

As mentioned in the Introduction (and as seen in Fig. 2), the amount of GABA in the crucian carp brain increases in response to anoxia (Nilsson, 1990), probably because the GAD-catalyzed conversion of glutamate to GABA is anaerobic and will continue during anoxia, while the breakdown of GABA is aerobic and will stop (Nilsson, 1990; Nilsson *et al.* 1990). Isoniazid, at a dose of $500\,\mathrm{mg\,kg^{-1}}$, was found to inhibit most of this increase, and to cause a marked inhibition of metabolic depression. A straightforward interpretation of these results is that the anoxia-induced increase in brain GABA concentration is an important mechanism behind metabolic depression, and that the inhibition of metabolic depression by isoniazid is a direct consequence of the ability of isoniazid to block the increase in brain GABA concentration. However, the situation may be somewhat more complicated because isoniazid and anoxia may affect different neuronal GABA pools. Recent results with the GABA synthesis inhibitor isoniazid and the GABA breakdown inhibitor gabaculine suggest that an inhibition of GABA synthesis will primarily decrease Ca^{2+}-dependent synaptosomal GABA release (probably from the vesicular pool), while an inhibition of GABA breakdown will mainly increase Ca^{2+}-independent release from synaptosomes (probably from the cytosolic pool) (Wood *et al.* 1988). Thus, it is probable that isoniazid treatment primarily decreases the GABA content of the vesicular pool, while anoxia increases the GABA content of the cytosolic pool. However, since we know nothing about the rate of exchange between these pools in crucian carp brain, it is difficult to judge whether a blockade of GABA synthesis will have more profound effects on GABAergic transmission than will a blockade of GABA degradation. Moreover, one may assume that GABA can act as an inhibitory neurotransmitter from whichever pool it is released, and it will therefore initiate metabolic depression.

GABA may not be the only metabolic depressant. The inhibitory amino acids glycine and taurine also increase in concentration during anoxia in the brains of crucian carp and/or freshwater turtles (Nilsson, 1990; Nilsson *et al.* 1990, 1991; Nilsson and Lutz, 1991), and their possible roles in metabolic depression remain to be studied. Moreover, I recently found that the rate of ethanol production in anoxic crucian carp increases after treatment with aminophylline, an adenosine receptor antagonist (Nilsson, 1991). At a dose of $75\,\mathrm{mg\,kg^{-1}}$, aminophylline caused a threefold increase in anoxic ethanol production, without affecting the rate of normoxic oxygen consumption. Taken together with the present results, this indicates that increased activation of both purinergic and GABAergic receptors is essential for metabolic depression. It might be expected that each of these inhibitory neurotransmitter/neuromodulator systems is responsible for only a part of the metabolic depression, and it may seem contradictory that both anti-GABAergic and anti-adenosinergic drugs can cause a threefold increase in anoxic metabolic rate, suggesting a virtually complete inhibition of metabolic depression with each group of drugs. However, when encountering a falling ambient oxygen level, the *Carassius* species appear to depress their metabolism in two steps. The

first step is that seen in Fig. 3, when the routine metabolic rate is lowered to about one-third of the initial value, reaching an oxygen consumption corresponding to the standard metabolic rate [about 50 mg $kg^{-1} h^{-1}$ in *C. auratus* at 20°C (Beamish and Mookherjii, 1964)]. The second step is the threefold decrease in heat production displayed by *Carassius* exposed to anoxia (Van Waversveld *et al.* 1989) after the standard metabolic rate has been reached following several days of starvation in darkness in a calorimeter. Nevertheless, the ethanol production rates measured in anoxic controls in this study closely correspond to those measured by Van Waversweld *et al.* (1989) in the calorimeter, showing that full metabolic depression is reached during anoxia regardless of the initial metabolic rate. Thus, *Carassius* species exposed to anoxia can be said to display a 3×3=ninefold change in metabolic rate if the total fall in energy utilization, from routine metabolic rate all the way to full anoxic metabolic depression, is taken into account. It is possible that only one of the GABAergic and purinergic systems needs to be functioning to accomplish most of the initial lowering of energy consumption, while both systems are needed for full metabolic depression. This possibility remains to be studied.

In conclusion, the results of the present study clearly indicate that the inhibitory neurotransmitter GABA is a mediator of metabolic depression in anoxic crucian carp. This mediatory role could be shared with other inhibitory neurotransmitters/neuromodulators such as adenosine, and the mechanisms behind metabolic depression may include an altered balance between inhibitory and excitatory neurotransmission. The results also suggest that full anoxic metabolic depression is not essential for short-term (<4 h) survival in anoxia.

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