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Effects of Arterially Infused Hydroalcoholic *Agaricus blazei* Extracts on Perfusion Pressure and Oxygen Uptake in the Bivascularly Perfused Rat Liver

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Abstract

In a preceding work we have reported experiments showing that an hydroalcoholic exctract of *Agaricus blazei* is able to exert purinergic effects in the isolated perfused rat liver when it is infused into the portal vein in monovascular perfusion (entry: portal vein; exit: hepatic vein). In the present communication we are presenting and discussing experiments done with the bivascularly perfused rat liver (entry: portal vein + hepatic artery; exit: hepatic vein) in order to verify if the hemodynamic effects also occur in the arterial bed. It was found that the *A. blazei* extract is also active when infused into the hepatic arterial bed, with differences in both sensitivity and nature of the effects on either perfusion pressure or oxygen consumption. Constriction of the arterial bed required much higher concentrations of the extract than the portal bed. The kinetics of the response was also different, with a biphasic instead of a monophasic response. These results provide a promising starting point for future studies aiming to bring to light more mechanistic details about these and possibly other effects.

Keywords

***Agaricus blazei*, Purinergic Action, Hemodynamics, Oxygen Uptake**

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1. Introduction

Agaricus blazei Murril ss. Heinemann, also called *Agaricus brasiliensis* Wasser & Didukh, is a basidiomycete popularly known in Brazil as Cogumelo do Sol and Cogumelo Piedade. It is widely used today as a natural therapy in the form of a medicinal extract against a variety of diseases [1] [2]. In a preceding work we have reported experiments showing that a hydroalcoholic extract of *Agaricus blazei* is able to exert purinergic effects in the isolated perfused rat liver when it is infused into the portal vein [3]. It is generally accepted that purinergic agents, mainly nucleotides and nucleosides, are important paracrine agents that can affect several aspects of metabolism and cellular functions in various tissues, from the brain to the liver [4]-[6]. Adenosine, for example, has several functions in the central nervous system ranging from inhibition of neurotransmission to neuroprotective actions under pathological conditions [5] [6]. These effects are mediated mainly by purinergic receptors, from which several types and subtypes were described [4].

There are several reports about physiologic and pharmacologic actions of mushroom extracts and it will not be a surprise if many of them turn out to be the consequence of an action of nucleosides and nucleotides. Consistently, in our preceding work several nucleosides and nucleotides were identified in the hydroalcoholic extract of *Agaricus blazei* [3]. The total content in nucleotides and nucleosides of the hydroalcoholic extract of *Agaricus blazei* reached 38.2 nmol/mg, and the most abundant components were adenosine (14.4 nmol/mg), uridine (10.82 nmol/mg) and guanosine (2.96 nmol/mg) [3]. Typical purinergic effects in the liver are transient increases in glycogenolysis and glycolysis and transient decreases in oxygen uptake [7]-[10]. Additionally, purinergic agents are also known to be active on hemodynamics, causing vasoconstriction or vasodilation, depending on the vascular bed [7]. In accordance with the presence of purinergic agents in the *Agaricus blazei* extract, the latter produced transient and Ca^{2+}-dependent increases in the portal perfusion pressure and transient increases in glycogenolysis and glycolysis [3].

In our preceding experiments the liver was perfused monovascularly, *i.e.*, only the portal vein was cannulated, a preparation that is usually used in metabolic studies. The liver has two entry vessels, however, as illustrated by **Figure 1**, and a more sophisticated preparation is the bivascularly perfused rat liver in which both vessels are cannulated [11]. This preparation has been successfully used for studying zonation of metabolism, but it is also useful for investigating hemodynamic changes in the arterial bed in addition to the hemodynamic alterations in the portal vein [11] [12]. The fact that the hydroalcoholic extract of *Agaricus blazei* is able to produce vasoconstriction in the portal bed raises the question if the same effects are also exerted in the hepatic arterial bed. In the present work the bivascularly perfused rat liver preparation was used to obtain a preliminary insight into this question. For this purpose, the *Agaricus blazei* extract was infused into the hepatic artery with simultaneous measurement of oxygen consumption by the whole organ and the arterial perfusion pressure.

2. Materials and Methods

2.1. Materials and Extract Preparation

The liver perfusion apparatus was built in the workshops of the University of Maringá. All chemicals were from the best available grade.

Fruiting bodies (basidiocarps) of *A. blazei* were obtained from a local producer in Maringá, PR, Brazil. The young basidiocarps (cap closed) were harvested and dried. The basidiocarps were milled until a fine powder was obtained. The samples (50 g) were extracted by stirring with 1000 mL of 70% ethanol (v/v) at room temperature and at 130 rpm for 3 h and filtered through Whatman no. 1 paper. The extraction was repeated two times. No increase in yield was achieved by further extractions. The combined filtrates were concentrated with a rotary vacuum evaporator at 40˚C to eliminate ethanol and finally freeze-dried. The freeze-dried powder (yield = 50%) was stored in freezer until use. The latter were directly dissolved in the perfusion medium at the desired concentration.

2.2. Liver Perfusion

Male albino rats (Wistar), weighing 190 - 220 g, were fed *ad libitum* with a standard laboratory diet (Purina®). For the surgical procedure the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). Hemoglobin-free non-recirculating bivascular liver perfusion was done either in the antegrade mode (entry via the portal vein plus hepatic artery and exit via the hepatic vein; see **Figure 1**). *In situ* perfusion was carried out, the

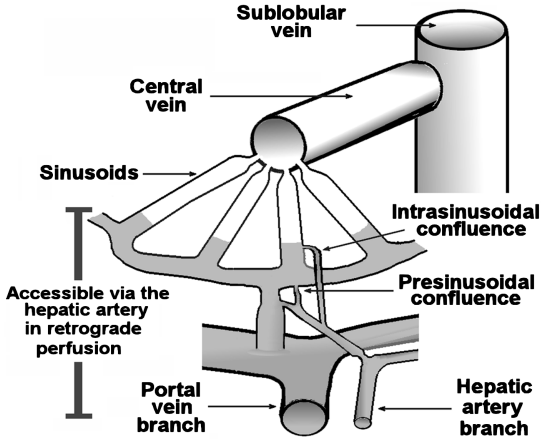

Figure 1. Diagram illustrating some of the main features of the hepatic microcirculation.

perfusate flow being provided by two peristaltic pumps. The perfusion fluid was Krebs/Henseleit bicarbonate buffer (pH 7.4) containing 25 mg% bovine serum albumin, saturated with a mixture of oxygen and carbon dioxide (95:5) by means of a membrane oxygenator with simultaneous temperature adjustment (37˚C). The composition of the Krebs/Henseleit-bicarbonate buffer is the following: 115 mM NaCl, 25 mM $NaHCO_3$, 5.8 mM KCl, 1.2 mM Na_2SO_4, 1.18 mM $MgCl_2$, 1.2 mM NaH_2PO_4 and 2.5 mM $CaCl_2$.

The surgical procedure described by Suzuki-Kemmelmeier *et al.* [13] was adopted. The final flow through both entry vessels was adjusted to 28 - 32 ml/min for the portal vein and 2 - 3 ml/min for the hepatic artery.

2.3. Measurement of Oxygen Consumption and Perfusion Pressure

The oxygen concentration in the outflowing perfusate was monitored polarographically, employing a teflon-shielded platinum electrode adequately positioned in a plexiglass chamber at the exit of the perfusate [14]. Oxygen consumption (expressed as μmol per minute per gram liver wet weight) was calculated from the difference between the constant input and the variable output, the total flow rate and the wet weight of the liver.

The perfusion pressure of the arterial bed was monitored by means of a pressure transducer (Hugo Sachs Elektronic-Harvard Apparatus GmbH, March-Hugstetten, Germany). The sensor was positioned near to the entry of the hepatic artery and the transducer was connected to a recorder. The pressure changes were computed from the recorder tracings and expressed as millimeters of mercury (mm Hg).

3. Results and Discussion

Two different experimental protocols were utilized in the present work. In the first one the *A. blazei* extract was infused into the hepatic artery at a rate that ensures a concentration of 400 mg/liter in the arterial bed. Since the ratio of arterial to portal flow is around 0.1, this concentration in the arterial bed will generate a sinusoidal concentration of 40 mg/liter (see **Figure 1** for details about the hepatic microcirculation). In the second protocol the *A. blazei* extract was infused into the hepatic artery at a concentration of 4000 mg/liter. This results in a mean concentration in the portal bed of 400 mg/liter, a concentration that produced an increase of about 8 mm Hg in the portal perfusion pressure (approximately 300% over the basal pressure) in addition to several metabolic effects [3]. Livers from fed rats were perfused with substrate-free perfusion fluid. Under these conditions the liver cells survive at the expense of endogenous glycogen (glycolysis) and fatty acids (β-oxidation) [14].

Figure 2 shows the results of the experiments in which the extract was infused at a concentration of 400 mg/liter, equivalent to an infusion rate of 0.15 $mg \cdot min^{-1} \cdot g^{-1}$. Zero time represents the point in time just after stabilization of oxygen consumption. The infusion of the extract was initiated 10 minutes after this time point. The arterial perfusion pressure responded with a small transient increase of 2.65 ± 0.77 mm Hg. This corresponds to a 5.3% increase above the basal pressure which is around 50 mm Hg in the isolated perfused rat liver [11] [12]. Oxygen uptake, however, did not experience any significant change over the entire period of the extract infusion (10 minutes).

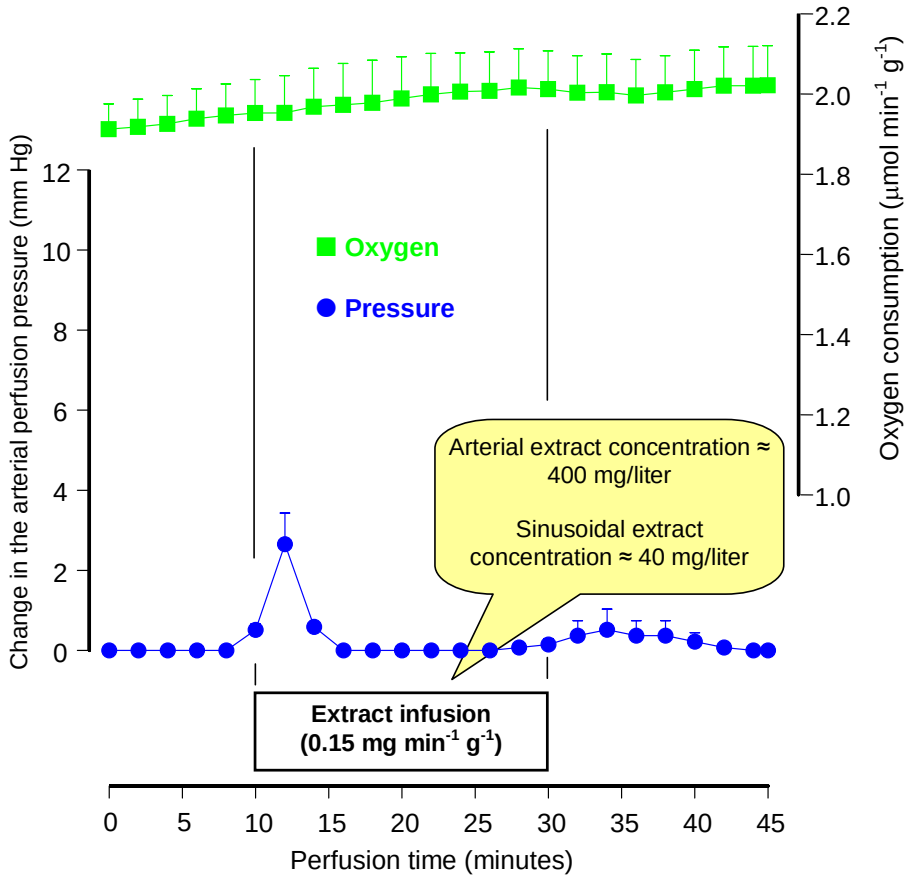

Figure 2. Arterial perfusion pressure and hepatic oxygen consumption: influence of the *Agaricus blazei* extract infused at a reduced rate. Livers were perfused bivascularly as described in Materials and Methods. The extract was infused from 10 to 30 minutes, as indicated. The perfusion pressure was monitored by means of a pressure transducer. Oxygen consumption was monitored polarographically. Data points ± mean standard errors are from three liver perfusion experiments.

Figure 3 shows the mean results of the experiments in which the extract was infused at a high concentration in order to achieve sinusoidal concentrations of 400 mg/liter. In this case the response of the arterial pressure was more pronounced and the kinetics was also different. There was an initial peak increment of 7.37 ± 1.46 mm Hg (14.6%) followed by a partial recovery. The latter, however, was immediately followed by a steady increase, almost linear with time, which reached 10.56 ± 1.42 mm Hg (21%) at the end of the extract infusion. The oxygen consumption also responded with an initial transient inhibition of maximally 0.77 µmol·min^{-1}·g^{-1}, followed by a recovery and a stimulation slightly above the basal rate.

The experiments indicate that the hepatic arterial bed also responds in a vasoconstrictive manner to the *A. blazei* extract. Its sensitivity, however, is less pronounced than that one found for the portal bed. Complexities arised, however, when high concentrations of the extract were infused. The kinetics of the phenomena suggest that there are two kinds of response: 1) a fast one, which is rapidly counter-regulated and which manifests also at low concentrations; and, 2) a slow one, which manifests only at high concentrations, but which is considerably more stable. About the agents that are responsible for both effects, only speculations can be done. A significant contribution of nucleosides and nucleotides can be expected, however, because these agents are well known for their hemodynamic effects.

The response of oxygen uptake, on the other hand, is somewhat surprising. Based on the experiments that were done by infusing 400 mg/liter directly into the portal bed of livers from fed rats one would expect a small inhibitory overshoot phenomenon followed by a constant and steady stimulation of oxygen uptake of approximately 0.61 µmol·min^{-1}·g^{-1} [3]. This stimulation was absent in the present experiments. This modified re-

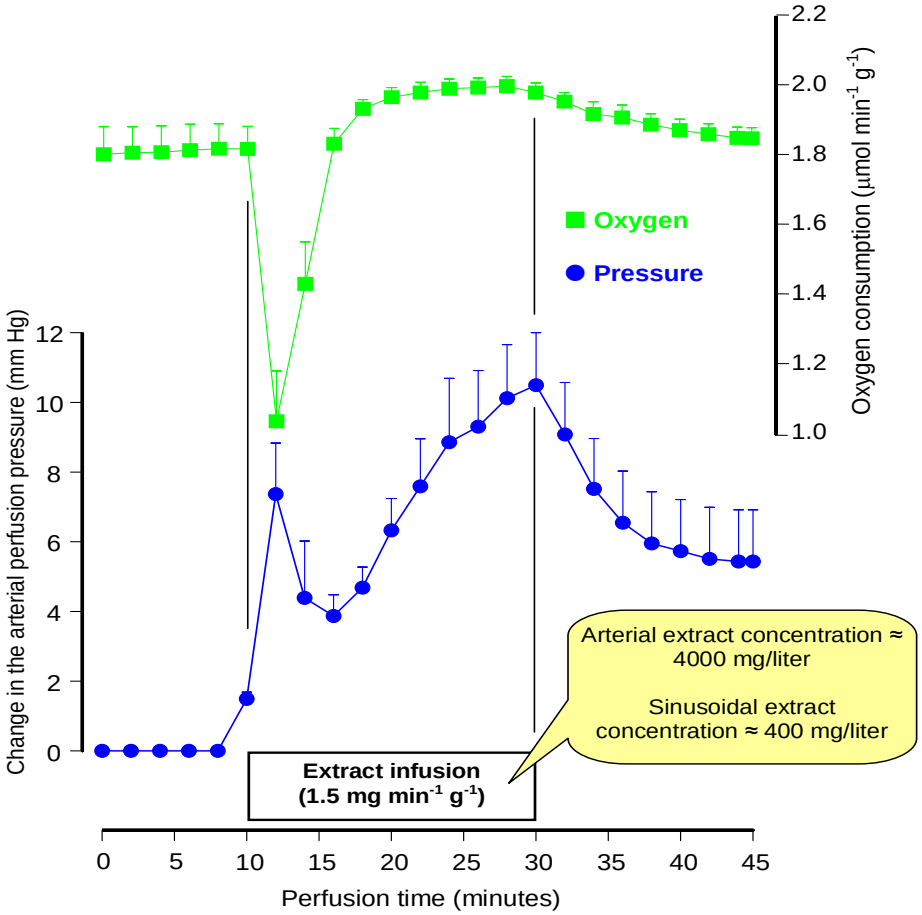

Figure 3. Arterial perfusion pressure and hepatic oxygen consumption: influence of the *Agaricus blazei* extract infused at a high rate. Livers were perfused bivascularly as described in Materials and Methods. The extract was infused from 10 to 30 minutes, as indicated. The perfusion pressure was monitored by means of a pressure transducer. Oxygen consumption was monitored polarographically. Data points ± mean standard errors are from three liver perfusion experiments.

sponse when the extract was infused into the hepatic artery suggests that the high extract concentration in the arterial bed could be inducing the release of substances that enhance the inhibitory effect of the extract so that stimulation does no longer occur. Favouring this hypothesis is the observation that the inhibitory effect when the extract is infused into the portal vein is completely abolished by inhibitors of eicosanoid synthesis and by the purinergic antagonist suramin [3]. Inhibition seems, thus, to be an indirect effect of components of the extract, dependent on the release of eicosanoids or other substances.

4. Conclusion

In conclusion it can be said that the *Agaricus blazei* extract is also active when infused into the hepatic arterial bed, with differences in both sensitivity and nature of the effects on either perfusion pressure or oxygen consumption. These results provide a promising starting point for future studies aiming to bring to light more mechanistic details about these and possibly other effects. It will be important to investigate how the arterially infused *A. blazei* extract affects glycogenolysis, glycolysis, gluconeogenesis, ureogenesis, and the redox state of the cytosolic NAD-NADH couple. When infused into the portal vein the *A. blazei* extract significantly modified all these parameters in addition to perfusion pressure and oxygen uptake. If the arterially infused *A. blazei* extract behaves differently in comparison with the portal infusion this could be an indication that its components can interact with specific targets in the arterial bed and induce the release of agents capable of modulating the metabolism of the liver parenchymal cells.

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