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D-Lactate inhibition of memory in a single trial discrimination avoidance task in the young chick

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Abstract

L-Lactate is a metabolite possibly able to meet some neuronal energy demands. However, a clear role for L-lactate in behaviour remains elusive. Administration of the inactive isomer D-lactate (1.75 mM; ic), immediately post-training, resulted in a persistent retention loss from 40 min post-training when used in conjuction with a single trial discrimination avoidance task designed for the young chick. Furthermore, 1 mM noradrenaline (ic) administered 20 min post-training overcame the retention loss induced by D-lactate. Although not directly demonstrated in the current study, it is plausible that D-lactate inhibited memory processing by competing with L-lactate for uptake into neurons. The time of onset of the retention loss induced by D-lactate is in accord with findings where the action of noradrenaline is inhibited. The successful challenge of D-lactate inhibition by a high concentration of noradrenaline may suggest a relationship by some unidentified mechanism.

Keywords: Lactate; Lactate shuttle hypothesis; Astrocyte; Glycogenolysis; Noradrenaline; Single trial discrimination avoidance learning task; Chick; Memory

1. Introduction

Traditionally, glucose has been considered the primary energy metabolite used by neurons (Chih, Lipton, & Roberts, 2001). In support of this: cerebral glucose utilisation, as measured by 2-deoxyglucose autoradiography, is correlated with brain activity (Sokoloff, 1983); glucose transporters (i.e., GLUT3) are found on neuronal membranes (Maher, 1995; Vannucci, Maher, & Simpson, 1997); and neuronal glycolytic enzyme activity increases when energy demands increase (Clarke & Sokoloff, 1998; Peng, Zhang, & Hertz, 1994). However, astrocytes are also well placed to take-up glucose from the cerebral vasculature. For example, GLUT1 glucose transporters are expressed on astrocytic endfeet which are found in close contact with cerebral capillaries (Leino, Gerhart, van Bueren, McCall, & Drewes, 1997; Morgello, Uson, Schwartz, & Haber, 1995; Pannese, 1994). Evidence that astrocytes take-up glucose also came from the fact that the brain's glycogen stores are localised almost exclusively in astrocytes (Fillenz, Lowry, Boutelle, & Fray, 1999). Yet when the product of astrocytic glycogenolysis was investigated L-lactate, not glucose, was demonstrated to be released from primary astrocytic cultures (Dringen, Gebhardt, & Hamprecht, 1993; Forsyth et al., 1996). This suggested that when released from astrocytes L-lactate may serve a similar role to glucose in the brain.

That L-lactate is a possible energy source for neurons comes about as studies investigating astrocytic monocarboxylates, including L-lactate, have noted monocarboxylate transporters (MCTs) on both astrocytes and neurons. Specifically, MCT1 is found on the surface of astrocytes (Chiry et al., 2006) while MCT2 is abundantly expressed on neuronal membranes (Pierre, Magistretti, & Pellerin, 2002; Pierre, Pellerin, Debernardi, Riederer, & Magistretti, 2000). Monocarboxylate transport is driven by concentration and proton gradients (Brooks, 2002) however

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pharmacokinetic studies suggest astrocytic MCT1 is suited to L-lactate release (Bröer et al., 1997; Volk, Kempski, & Kempski, 1997) while neuronal MCT2 is suited to L-lactate up-take (Bröer et al., 1998; Bröer et al., 1999; Halestrap & Price, 1999). In this way it is possible for L-lactate to be shuttled from astrocytes to neurons.

In addition, L-lactate can also be metabolised by neurons (Bouzier-Sore et al., 2006; Itoh et al., 2003) being rich in the lactate dehydrogenase (LDH) type 1 subunit. Although the exact conditions under which L-lactate may be produced or consumed by LDH-catalysed reactions remains debated (Cerdan et al., 2006; Chih & Roberts, 2003; Schurr, 2006), this suggests neuronal LDH favours the conversion of L-lactate to pyruvate (Bittar, Charnay, Pellerin, Bouras, & Magistretti, 1996; Pellerin et al., 1998; Tsacopoulos & Magistretti, 1996). It has even been demonstrated that L-lactate (2–20 mM) can sustain synaptic function in the absence of glucose using rat hippocampal slices (Schurr, West, & Rigor, 1988) and that lactate is as effective as glucose as a metabolic substrate for fundamental neuronal processes such as activity-dependent synaptic vesicle cycling in cultured cortical neurons (Morgenthaler, Kraftsik, Catsicas, Magistretti, & Chatton, 2006).

From evidence such as this Pellerin and Magistretti (1994) developed a new and controversial (Chih & Roberts, 2003; Dienel & Cruz, 2004; Hertz, 2004) hypothesis of brain energy metabolism which proposes that L-lactate is an important neuronal energy metabolite. Known as the "astrocyte-neuron lactate shuttle hypothesis", or more simply as the "lactate shuttle hypothesis", it proposes that glucose is taken-up by astrocytes, metabolised to L-lactate, released and transported into neurons to be used as an alternative energy metabolite to glucose (Pellerin & Magistretti, 2003).

While evidence for the role of L-lactate *in vivo* is scarce, nuclear magnetic resonance (NMR) spectroscopy using rodents has allowed the observation of L-lactate metabolism in the brain. Studies using this technique have supported the role of L-lactate as an energy metabolite, suggesting that glucose may be metabolised in astrocytes and that L-lactate can be consumed in neurons (Bouzier et al., 2000; Garcia-Espinosa et al., 2004; Hassel & Brathe, 2000). However, one shortcoming of such studies is that NMR spectroscopy does not specifically confirm that astrocytic L-lactate is shuttled from astrocytes to neurons. Furthermore, although NMR spectroscopic studies hint at the role of L-lactate *in vivo*, they do not conclusively demonstrate its importance in behaviour.

In contrast, pharmaco-behavioural investigations provide an excellent means to determine if L-lactate has a significant role in brain activity leading to behaviour. Although Dong et al. (2003) did not observe changes to L-lactate production in the hippocampus of rats trained using a shock-based operant task coupled to microdialysis, they did observe a reduction in L-lactate for control rats which were repeatedly shocked. However, their findings are difficult to interpret (Uehara, Sumiyoshi, Matsuoka, Itoh, & Kurachi, 2006) as the type, strength and frequency of reinforcement used may be an issue.

In contrast, the present study employed a single trial discrimination avoidance task developed for the young chick. Not only is this task considered to be ecologically valid, but it also uses a single training experience such that the time of learning can be clearly identified. The current task is also advantageous as it builds upon past studies investigating aspects of memory processing potentially related to the role of L-lactate. For example, astrocytic glycogenolysis is an important process leading to the formation of long-term memory by 60 min post-training (Gibbs, Anderson, & Hertz, 2006; O'Dowd, Gibbs, Ng, Hertz, & Hertz, 1994; O'Dowd, Gibbs, Sedman, & Ng, 1994) and is known to be stimulated by noradrenaline (O'Dowd, Barrington, Ng, Hertz, & Hertz, 1995; Subbarao & Hertz, 1990). Noradrenaline is similarly critical for the formation of long-term memory (Crowe, Ng, & Gibbs, 1990; Crowe, Ng, & Gibbs, 1991).

Based upon the evidence above, the present study sought to investigate whether the inactive D-lactate isomer will inhibit memory, possibly through competitive uptake with L-lactate.

Such results may suggest, albeit indirectly, that L-lactate is involved in memory processing. As L-lactate is synthesised in astrocytes it may be speculated that D-lactate will inhibit memory at a time consistent with astrocytic glycogenolysis.

2. Materials and methods

2.1. Animals

Each week, day-old white-Leghorn × black-Australorp chicks were obtained from a local hatchery. Chicks were divided randomly between those to be used that day or the following day. If needed for the following day's experiments chicks were housed in a purpose-built brooder (2.5 × 1.5 m). Crushed poultry food and water were supplied *ad libitum* to chicks housed in the brooder and heating was maintained at 42 °C beneath two suspended heat lamps.

All behavioural experiments were conducted in a purpose-built behavioural laboratory which could accommodate ⩽120 chicks on any experimental day. The laboratory temperature was maintained between 26–29 °C using a wall mounted reverse cycle air conditioner. Additional warmth was provided by a series of single 15 W light globes suspended above each pair of chicks housed in pens.

All procedures were approved by the Monash University Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition, 2004). Chicks were housed in randomly determined pairs in wooden pens (18 × 25 × 20 cm) to avoid isolation stress which is known to affect the memory trace (DeVaus, Gibbs, & Ng, 1980). Ten pairs of chicks represented one data-point. Prior to occupation, the floor of each pen was scattered with crushed poultry food. For identification, one chick per pair was marked with a black felt-tip pen on the back of the head. Chicks were left undisturbed for approximately 30 min before the task began to allow for acclimatisation.

2.2. Task procedure

Chicks were trained using the strongly reinforced variant of the single trial discrimination avoidance task popularised by Gibbs and Ng (1977) and described elsewhere in detail (Gibbs, 1991). In brief, the task is a bead