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Effects of artificial rearing on contractile properties of genioglossus muscle in Sprague–Dawley rat

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ABSTRACT

Mammals suckle from a nipple during the early neonatal period to obtain nourishment. The genioglossus muscle helps position and move the tongue for efficient suckling. The purpose of this study was to examine the contractile properties and myosin heavy chain (MHC) phenotype of the genioglossus following an early period of artificial rearing, which reduced nutritive suckling. Beginning at 3 days of age, rats were fed via gastric cannula until postnatal day 14 (P14). At P14, artificially reared rat pups were either allowed to grow to postnatal day 42 (P42) or anaesthetised and prepared for experimentation. Comparisons were made between artificially reared and dam reared groups at P14 and P42. At P14 maximum tetanic tension and fatigue index were lower in the artificially reared group than the dam reared group. By P42, artificially reared rats had a higher fatigue index and lower percentage of MHCIIa than dam reared rats. The artificial rearing technique employed in this study was adequate to produce chronic changes in fatigue resistance and MHC distribution in genioglossus muscle of rat; the changes observed here may be similar to changes that occur in premature human infants requiring early artificial feedings.

1. Introduction

Pre-term and perinatal infants requiring nutritional and/or respiratory support often spend the first days or weeks of life in the neonatal intensive care unit (NICU). Frequently these infants display feeding[1,2] and motor speech delays.[3,4] Healthy full term infants usually suckle soon after birth while pre-term and medically compromised infants frequently require life saving interventions, such as oro-tracheal intubation or naso-gastric tube feedings, which may interfere with suckling.[2,5] Hawdon et al.[2] found that pre-term human infants requiring NICU placement showed abnormal feeding patterns at 40 weeks postmenstrual age, and at 6 months and 1 year of age. Additionally, Luoma et al.[6] found that neurologically normal pre-term infants born before 32 weeks gestational age still had language production problems at 5 years of age, while Jennische and Sedin[4,7] found that several aspects of speech and language were delayed in 6.5-year-old pre-term children compared to their full-term cohorts. According to the National Center for Health Statistics pre-term births (births before the completion of the 37th gestational week) accounted for 12.1% of all live births in 2002, up from 11.9% in 2001, and a 29% increase since 1981.[8] The financial burden associated with caring for these children has increased proportionally.

The oro-motor dysfunction problems reported above may be due, at least in part, to the disruption of the normal

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development of the hypoglossal motor system. This study sought to disrupt nutritive suckling behaviour in otherwise healthy developing rats through an altered activity model. While this model prevented nutritive suckling, the animal was able to move all oral structures freely. In fact, rat pups were frequently observed sucking on bedding and often made vocalizations.

Altered activity is an example of an environmental factor in which a researcher attempts to influence the normal use of a muscle or muscle group. Examples of altered activity include hind limb suspension,[9–14] microgravity,[13,15–17] and immobilization.[18] In general, altered activity in neonatal rats has been shown to cause slow twitch muscles, such as the soleus, to contract faster[10] and to retard slow twitch muscle fibre growth.[9,11] In addition, the normal conversion from fast to slow phenotypes stops.[9–11] Altered activity during development also causes fast twitch muscles, such as the extensor digitorum longus and plantaris, to contract faster.[19] Fast twitch muscle fibre growth continues at near normal rates, and the differentiation of fibre types from developmental to adult isoforms slows.[11]

The artificial rearing methods used in this study, in which young rat pups are fed via an indwelling gastric cannula so that opportunity for nutritive suckling is bypassed, has been employed previously in this lab. Kinirons et al.[20] found that artificial rearing from P4 to P14 produced long term muscle phenotype changes in a tongue retractor muscle, styloglossus.

The hypoglossal motor system, which functions to move the tongue, comprises three extrinsic (muscles that originate from a boney attachment and insert on the body of the tongue) muscles (hyoglossus, styloglossus and genioglossus) and four intrinsic (origin and insertion in tongue body) muscles. Here we focus exclusively on the genioglossus muscle which is responsible for depression and protrusion of the base of the tongue and is innervated by the medial branch of the hypoglossal nerve (CNXII). The neurons driving genioglossus muscle activity are located in the ventral compartment of the hypoglossal nucleus.[21,22]

In humans the coordination required to suckle from a nipple is generally present between the 32nd and 34th gestational week.[23] Nutritive suckling begins with nipple attachment. In a rat model Westneat and Hall found that nipple attachment lasts 5–10 s and relies heavily on the alternating contraction of the masseter and the genioglossus and digastric muscles.[24] After nipple attachment, rhythmic suckling begins and is characterized by increases and decreases in intra-oral pressure[24] (as measured by an artificial nipple with a force transducer mounted at the tip). Rat studies, using electromyography,[24] and human studies, using ultrasound,[23] show that changes in intra-oral pressure result from tongue movement. Tongue depression results in a decrease in intra-oral pressure and is brought about by the contraction of the genioglossus while increases in intra-oral pressure result from tongue elevation as the genioglossus relaxes and the masseter contracts.[23,24] Decreases in intra-oral pressure draw milk into the oral cavity while increases in intra-oral pressure slow the flow of milk so that the animal can swallow.[23]

The timing of the onset of nutritive suckling may be critical in rats. During the first 2 weeks of life, rat genioglossus motoneuron dendrites undergo a period of simplification followed by a 2 week period where dendritic surface area doubles.[25] Alterations in oromotor activities during this time may have a profound impact on later development of oral motor skills. Therefore, the purpose of this study was to examine the contractile properties and MHC composition of the genioglossus muscle in rats deprived of suckling during the early neonatal period and to compare these results to dam reared rats.

2. Materials and methods

All procedures and protocols were approved by Virginia Commonwealth University's Institutional Animal Care and Use Committee. Animals were housed on a 12 h light/dark cycle and control rats had unrestricted access to the dam.

A total of 40 young Sprague–Dawley rats underwent experimentation. The rats were placed into one of two groups based on rearing method, a dam reared group and an artificially reared group. The rat pups in the artificially reared group underwent gastric cannulation on P3 and were fed an artificial formula (described below) through their cannulas from P3 to P14. Rat pups in the dam reared group were raised by the dam in the usual manner. On P14, 10 rats from the dam reared group and 10 rats from the artificially reared group were anaesthetized and prepared for physiological experimentation, the remaining 10 dam reared rats were left with dams. The 10 remaining artificially reared rats had their ears marked for identification and were placed in a cage with a lactating dam and age matched dam reared rat pups until P21. On P21, rat pups were separated into groups of 3–5 rats per cage with the dam removed. On P42, the 10 remaining rats from each rearing group were prepared for experimentation.

2.1. *Artificial rearing*

The artificial rearing methods employed in this study were developed by Hall[26] and have been used previously in this lab.[20] On P3 rat pups were anaesthetized with isoflurane and cannulated intragastricly. Cannulation involved the insertion of a 25 cm length of polyethylene tubing, with an inside diameter of 0.28 mm (PE-10), into the rat's stomach; one end of the cannula was melted so that it creates a flange. Cannulation was accomplished by inserting a lightly lubricated 6.5 cm piece of polyethylene tubing with an inside diameter of 0.58 mm (PE-50) in to the rat's oral cavity and carefully sliding it down the esophagus and into the stomach. Once placement of this lead tube was confirmed, a 31-gauge stainless steel wire stylet was fed through the lead tube and the abdominal wall was punctured. Next, the lead tube was removed and the unflanged end of the cannula was friction fitted onto the oral end of the wire stylet and the wire stylet, with the cannula attached, was pulled through the abdominal wall so that the flanged end of the cannula formed a seal with the internal wall of the stomach. The cannula was secured by placing washers made from 1 cm sections of PE-50 against the external abdominal wall. All instruments, cannulas, lead tubes and wire stylets were sterilized immediately prior to use. Topical antiseptic was applied to the cannula's exit site immediately after cannulation. The cannulated rats were