Congenital hypothyroidism alters formalin-induced pain response in neonatal rats

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ABSTRACT

The present study designed to investigate the development of nociceptive circuits upon formalin-induced pain in congenital hypothyroid pups during the first three postnatal weeks. Following induction of maternal hypothyroidism, the offspring pups were received right intraplantar injection of different formalin concentrations at 7, 15, and 23 days of age. Significant reduction in weight gain was observed in PTU-treated offspring from postnatal days 15 up to 23 (P < 0.001). No difference was observed between normal and hypothyroid PND7 pups in total pain intensity score with 0.3% solution of formalin. However, normal pups showed higher total pain score (P < 0.01) during the first phase of 1% formalin injection. PND15 normal pups showed a biphasic pain response with a concentration of 2% formalin injection. Obvious persistence of higher pain intensity was observed in hypothyroid pups after interphase through the 2nd phase (P2) and recovery phase (P3), (P < 0.001). PND23 hypothyroid rats showed slightly biphasic pattern of pain behavior with persistence of lower pain intensity during P2 (2.5% formalin, P < 0.05), (10% formalin, P < 0.001) without any further decline during P3 (P < 0.01, P < 0.001 respectively). In general, the number of flexes + shakes in hypothyroid pups was higher than normal pups in both the early and late phases of the test. Licking activity was intensively expressed only in normal pups during phase 2 at the age of 23 days. In contrast to acute pain, hypothyroidism results to pain hypersensitivity in two weeks old rats whereas weaned rats were hypersensitive to tonic nociceptive stimulation without showing the subsequent recovery phase.

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1. Introduction

In the rat, structural maturation and functional elaboration of nociceptive processes continue well into the postnatal period (Barr, 1998; Alvares and Fitzgerald, 1999; Walker et al., 2003). Postnatal changes in the somatosensory system determine pain sensitivity and sensory processing at each developmental stage (Fitzgerald and Jennings, 1999). In rodents induction of hypothyroidism during the prenatal period until weaning results in a number of effects on the development and function of the central and peripheral nervous system (Glauser and Walter, 1997; Thompson and Potter, 2000). Therefore, congenital hypothyroidism may alter the maturation of somatosensory pathways, changing nociceptive responses to injury induced pain processing. Some animal studies have tried to establish the role of thyroid hormone on nociception. Bruno et al. (2005a) reported that hyperthyroidism reduces the tail-flick pain threshold at an early postnatal age. Then they showed that the hypothyroidism decreases the pain sensitivity in newborn and old aged rats, but increases the nociceptive response in young adult rats (Bruno et al., 2005b). Edmondson et al. (1990) found that the hyperthyroid mice demonstrate greater sensitivity to thermal noxious stimulation. The above findings suggested that thyroid hormone plays a key role in acute (phasic) pain in response to brief mechanical and thermal stimuli. However it is believed that tonic pain such as formalin test is modulated differently in the central nervous system (Tjolsen et al., 1992). This test is commonly used as a valid model for clinical pain and allows the separation of early acute phase and next tonic phase and also the isolation of an active inhibitory phase (Gaumond et al., 2002). The first phase has been viewed as direct chemical activation of nociceptors in the periphery, then descending inhibition (interphase) and then the second phase is related to the sensitization of central nociceptive neurons due to peripheral inflammation as well as ongoing activity of primary afferents (Tjolsen et al., 1992; Abbott et al., 1995). The nociceptive responses of pre-weaned rat pups to subcutaneous injection of varying formalin concentrations change during development (Guy and Abbott, 1992; Teng and Abbott, 1998; Barr, 1998). Thus, the sensitivity to formalin pain is an expression of neuronal plasticity in pain processing as the nervous system.
develops (Woolf and Salter, 2000). In addition, maturation of the nervous system of a newborn rat is developmentally similar to a third trimester human fetus (Anderson, 2001). The congenital hypothyroidism is the most prevalent endocrine disorder in the newborn and affects 1 in 3000–4000 newborns (Gruters et al., 2003). No study has been developed as yet for assessing tonic and long-lasting pain behaviors in hypothyroid neonate rats during the human developmental equivalent of third trimester. In the present study, we therefore investigated the pain sensitivity and responsiveness at different stages of development in congenital hypothyroid neonates using the formalin test, a model of both acute and tonic pain.

2. Materials and methods

2.1. Animals and experimental hypothyroidism

Sprague-Dawley (Pasteur’s institute, Tehran, Iran) female rats were mated overnight and the following morning was set as the first day of gestation. Chemical treatment started from the 16th day of gestation and continued until the 23rd postnatal day. Dams were received 0.005 g/100 ml of anti-thyroid drug propylthiouracil (PTU, Iran hormone) in their drinking water. This concentration represents the same amount of PTU which was received by the pups during suckling period (Blake and Henning, 1985). Normal pups received tap water. Pups found at either time were termed 0 days of age. After parturition litters were culled to 8–10 pups on postnatal day 1 for each dam. Ten intact and 10 hypothyroid rat pups were tested at 7, 15 or 23 days of age. Each pup was tested only once. All experiments were performed according to the guidelines for animal care set forth by IASP.

2.2. Agents

Formaldehyde and 0.9% saline were used to prepare formalin solutions. One percent formalin was made by mixing 0.1 ml formaldehyde with 9.9 ml saline. Low and high concentrations of formalin were adjusted for each group according to Teng and Abbott (1998) and were as follows: PND7 pups were tested with 0.3% and 1% formalin, PND15 pups with 0.5% and 2%, and PND23 pups with 2.5% and 10%. Volume of injections was 10 μl for all groups.

2.3. Formalin test

Our studies focused on the right hind paw. Since the paw is most accessible in the infant rat, and lots of efforts have been made on inflammation of the paw we limited our model to that limb. Formalin was injected subcutaneously into the plantar surface of right hind paw while the rat was restricted. Following the formalin injection all pups were placed immediately in a bare plastic chamber. The pain behavior of the pups was scored every 15 s for a 60 min test, using the time-sampling method (Abbott et al., 1999). In addition, the number of flexes + shakes and licks were counted during first phase (P1) (0–5 min after injection), interphase (Int P) (5–10 min), second phase (P2) (10–35 min) and the onset of recovery phase (P3) (35–60 min). To prevent the stress of a novel environment, all rats were habituated to the formalin test chambers 30 min prior to formalin injection (Guy and Abbott, 1992). The formalin tests were performed in clear plastic boxes with a mirror placed underneath at a 45° angle to allow an unimpeded view of the animals paw. Two rats were observed simultaneously (one rat per box).

2.4. Statistics

The behavioral data were averaged into 5 min bins to decrease minute by minute variability. Statistical significance for each phase was analyzed with two tailed Student t-test using SPSS software. For all tests the level of significance was set at p < 0.05. All data are reported as mean ± S.E.M.

3. Results

In this study PTU-treated and normal pups were weighted at 7, 15 and 23 days after birth (Fig. 1). The hypothyroid pups showed about 50% reduction in weight gain at the time of weaning. PTU-treated pups displayed blunt snouts, unfolded ears and rounded bodies; and compared to the normal pups eye opening was delayed for 2 days (Sawin et al., 1998). Nevertheless, the hypothyroid pups were able to perform the pain behaviors including limb favoring, flexion, shaking and licking as of day 7 of postnatal life.
contrast for 1% formalin concentration the number of flexes + shakes was not increased in hypothyroid pups in comparison to normals (Fig. 5). The licking behavior was observed rarely in both groups.

3.2. PND15 pups

The injection of two different concentrations of formalin induced significantly higher nociceptive score, during all the phases in hypothyroid pups compared to normal pups (Fig. 3). However the most prominent behavioral pain response was flexion + shaking of the limb on the injected paw; and the quantity of this behavior drops to half and one/third in hypothyroid and normals respectively in comparison to 7-day-old pups. It should be noted that the number of flexes + shakes are increased to three folds with higher formalin concentration in hypothyroid pups. The number of licking behavior was very low and not significant in both groups (Fig. 5, PND15).

3.3. PND23 pups

In normal pups, injection of two different formalin concentrations generated the typical biphasic nociceptive response, consisting of an early excitatory phase (P1), followed by a period of quiescence (interphase) (IntP), then a resumption of nociceptive behavior (second phase) (P2) and ultimately a significant decline in responding during the last minutes of the session (recovery phase) (P3). In general the nociceptive behavior was markedly attenuated during the second phase (P2) in hypothyroid pups, indicating a pronounced hyposensitivity to formalin-induced noxious stimulation compared to normals. Moreover, the hypothyroid pups showed significantly and continuously a higher nociceptive score in the last minutes of the second phase or recovery phase (P3). During the same period of the test, the pain response decreased to near zero level in normal pups. Indeed, hypothyroid pups displayed a pattern of pain response similar to 15-day-old normal pups injected with 2% formalin concentration (see Fig. 4). No significant differences between hypothyroid and intact animals were observed in the total nociceptive score for interphase (Fig. 4). The number of licks counted in normal pups was significantly higher than hypothyroid pups during the first and second phase of the test. Injection of different formalin concentrations produced prominent and persistent limb flexes + shakes in the hypothyroid pups. In the last part of the test (P3) the mean number of flexes + shakes was significantly higher than normals in hypothyroid pups (Fig. 5, PND23). In addition, they showed a significant reduction of licking behavior during the second phase (Figs. 4 and 5 PND23).

4. Discussion

Reflecting our previous reports and those of others, induction of congenital hypothyroidism caused a severe decline in body weight starting from the second postnatal week (Blake and Henning, 1985; Sawin et al., 1998; Behzadi and Ganji, 2005). By the first week of life the pain response was monophasic with no significant difference in the total nociceptive score between normal and
hypothyroid pups. The data is in consistence with that of Guy and Abbott (1992) and Teng and Abbott (1998) in newborn to one-week-old normal pups. In regard to formalin specific behaviors (flexes + shakes and licks) the sum of ipsilateral limb flexes + shakes was significantly higher in hypothyroid pups for lower formalin concentration. At the 15th postnatal day the total number of flexes + shakes of the injected paw were reduced compared to 7-day-old pups. Yet, it was significantly elevated in relation to formalin concentrations for hypothyroid pups. These behavioral reflexes are considered the most sensitive and specific measures of pain (Guy and Abbott, 1992). It has been shown that the opioid analgesia attenuates these specific pain behaviors in 2-week-old rats (Zissen et al., 2006). In accordance, the maturation of C-fiber input to the spinal cord is completed around the second postnatal week (Jenning and Fitzgerald, 1998). Higher pain sensitization was also observed in 15-day-old hypothyroid pups in the first phase. After the initial phase, the quiescent interphase was observed in both groups suggesting the appearance of a clear biphasic response especially for higher concentration of formalin (Teng and Abbott, 1998; Fitzgerald and Jennings, 1999). In contrast to normal pups, after the second phase, the nociceptive score were persisted in hypothyroid pups. Indeed, the 15-day-old hypothyroid pups showed increased nociceptive sensitization during the first phase, second phase and the subsequent recovery phase. In contrast, Bruno et al. (2005b) reported a hypoalgesic response in tail-flick test in 2-week old rats submitted to neonatal hypothyroidism. An imperfect pattern of biphasic response was also appeared in 23 days old hypothyroid pups. Unrelated to formalin concentrations, they also showed more sensitive pain response in the first phase. Typical biphasic nociceptive response was observed in normal weaned pups similar to that of adult rats. Using different ranges of formalin concentrations Teng and Abbott (1998) reported the same results in weaned rat pups. The biphasic pattern is thought to reflect the effects of acute chemical stimulation followed by the development of pain generated in injured and inflamed tissue (Guy and Abbott, 1992). The first phase of formalin response is

![Fig. 5. Histograms present the total number of flexes + shakes and licks in each phase for various formalin concentrations (0.3 and 1% for PND7; 0.5 and 2% for PND15; 2.5 and 10% for PND23). Note the higher number of flexes + shakes and lower number of licking activity in 15- and 23-day-old hypothyroid pups during all phases. P1; phase1, Int P; interphase, P2; phase2, P3; recovery phase. Data are mean ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.001.](image-url)
characterized by the direct activation of primary afferents and release of excitatory neurotransmitters into the dorsal horn of the spinal cord. Then active descending inhibition (interphase) and second phase follows that mediated by ongoing primary afferent activity coupled with central sensitization, after which the pain response declines over the succeeding hours (recovery phase) (Henry et al., 1999; Gaumond et al., 2002). The hyperalgesic first phase and the active interphase were produced at all ages (Walker et al., 2007) in both normal and hypothyroid pups (present results). The hypoalgesic second phase in hypothyroid weaned pups with persistent higher number of limb flexion (a predominantly spinal reflex) in comparison with the reduced supraspinally mediated licking response, suggests the immaturity of spinal and/or supraspinal mechanisms required for C-fiber-induced secondary hyperalgesia (Porro et al., 2003; Walker et al., 2007). In contrast, Bruno et al. (2005b) reported increased sensitivity (hyperalgesia) to tail-flick test in young hypothyroid rats. Reduced sensitivity to nociception of Ts65Dn mice as a model of Down syndrome on the formalin and tail-flick tests was observed by Martinez-Cue et al. (1999). Moreover, Fmr1KO mice (lacking the fragile X mental retardation protein) show decreased responses to ongoing nociception (phase2, formalin test) (Price et al., 2007).

The present results may suggest the fundamental role of thyroid hormone on the neuroplastic mechanisms involved in the mature appearance of nociceptive circuits, and the importance of the most appropriate type of analgesics that relieve the effects of the pain exposure in hypothyroid newborns.

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