Liver-derived IGF-I regulates exploratory activity in old mice

Johan Svensson, Bo Söderpalm, Klara Sjögren, Jörgen Engel, and Claes Ohlsson. Liver-derived IGF-I regulates exploratory activity in old mice. Am J Physiol Endocrinol Metab 289: E466–E473, 2005. First published April 19, 2005; doi:10.1152/ajpendo.00425.2004.—Growth hormone (GH) replacement in hypopituitary patients improves well-being and initiative. Experimental studies indicate that these psychic effects may be reflected in enhanced locomotor activity in mice. It is unknown whether these phenomena are mediated directly by GH or by circulating IGF-I. IGF-I production in the liver was inactivated at 6–10 wk of age (LI-IGF-I/+/− mice), resulting in an 80–85% reduction of circulating IGF-I, and, secondary to this, increased GH secretion. Using activity boxes on three different occasions during 1 wk, 6-mo-old LI-IGF-I/−/− mice had similar activity levels, and 14-mo-old mice had a moderate but significant decrease in activity level, compared with control mice. At 20 mo of age, the LI-IGF-I/−/− mice displayed a more prominent decrease in activity level with decreased horizontal activity throughout the test period, and at day 1, there were several signs of an altered habituation process with different time patterns of locomotor activity and horizontal activity compared with the control mice. At days 3 and 5, rearing activity was lower in the 20-mo-old LI-IGF-I/−/− mice. Anxiety level was unaffected in all age groups, as measured using the Montgomery’s elevated plus-maze. In conclusion, old LI-IGF-I/−/− mice displayed a decrease in both horizontal and rearing (exploratory) activity level and an altered habituation process. These results indicate that liver-derived IGF-I mediates at least part of the effects of GH on exploratory activity in mice.

Circulating insulin-like growth factor I; activity box; locomotor activity; rearing activity; habituation

Svensson, Johan, Bo Söderpalm, Klara Sjögren, Jörgen Engel, and Claes Ohlsson. Liver-derived IGF-I regulates exploratory activity in old mice. Am J Physiol Endocrinol Metab 289: E466–E473, 2005. First published April 19, 2005; doi:10.1152/ajpendo.00425.2004.—Growth hormone (GH) replacement in hypopituitary patients improves well-being and initiative. Experimental studies indicate that these psychic effects may be reflected in enhanced locomotor activity in mice. It is unknown whether these phenomena are mediated directly by GH or by circulating IGF-I. IGF-I production in the liver was inactivated at 6–10 wk of age (LI-IGF-I/+/− mice), resulting in an 80–85% reduction of circulating IGF-I, and, secondary to this, increased GH secretion. Using activity boxes on three different occasions during 1 wk, 6-mo-old LI-IGF-I/−/− mice had similar activity levels, and 14-mo-old mice had a moderate but significant decrease in activity level, compared with control mice. At 20 mo of age, the LI-IGF-I/−/− mice displayed a more prominent decrease in activity level with decreased horizontal activity throughout the test period, and at day 1, there were several signs of an altered habituation process with different time patterns of locomotor activity and horizontal activity compared with the control mice. At days 3 and 5, rearing activity was lower in the 20-mo-old LI-IGF-I/−/− mice. Anxiety level was unaffected in all age groups, as measured using the Montgomery’s elevated plus-maze. In conclusion, old LI-IGF-I/−/− mice displayed a decrease in both horizontal and rearing (exploratory) activity level and an altered habituation process. These results indicate that liver-derived IGF-I mediates at least part of the effects of GH on exploratory activity in mice.

Circulating insulin-like growth factor I; activity box; locomotor activity; rearing activity; habituation

Address for reprint requests and other correspondence: J. Svensson, Research Centre for Endocrinology and Metabolism, Göteborg University Hospital; and D. Department of Pharmacology, Göteborg University, Göteborg, Sweden

Submitted 16 September 2004; accepted in final form 18 April 2005

HYPOPTIUTARY ADULT HUMANS with growth hormone (GH) deficiency have a low serum IGF-I level and decreased quality of life (2, 5, 11, 23). These patients suffer from social isolation, subnormal cognitive function, tiredness, lack of initiative and concentration, irritability, reduced physical and mental drive, decreased vitality, and difficulties in coping with stressful situations (2, 5, 8, 11, 23). GH replacement in hypopituitary adults improves serum IGF-I level and quality of life (5, 7, 10, 15, 26). Moreover, in normal aging, serum IGF-I decreases in parallel with other age-related changes, such as decreased activity level and increased social isolation (19). The decrease in serum IGF-I with age could therefore be important for age-related changes in activity level and behavior.

Transgenic mice with general overexpression of GH under control of the metallothionein promoter (Mt-bGH) display increased spontaneous locomotor activity and an altered habituation process (32). The Mt-bGH mice also show an increased stimulatory response to amphetamine, suggesting sensitization of the mesocorticolimbic dopamine system, a part of the brain reward system that has been linked to anhedonia/hedonia and psychic drive. In addition, the Mt-bGH-transgenic mice show altered dopamine metabolism and alterations of the neurochemistry of the brain serotonin (5-hydroxytryptamine), a neurotransmitter that has been implicated in the regulation of mood (24) and locomotor activity (25). In another transgenic mouse strain, in which GH is overexpressed only in the central nervous system under control of the glial fibrillary acidic protein promoter (GFAP-bGH mice), locomotor activity is similar to that in control mice (6). Therefore, the effects of GH on locomotion and behavior in mice appear to be mediated by a peripheral factor (6).

IGF-I is one candidate that could mediate the effects of GH on locomotion and behavior. The major part of serum IGF-I is liver derived (31, 38). The serum levels of IGF-I are regulated mainly by GH (5) but also by other factors such as food intake, exercise, and age (9, 16, 19). Circulating IGF-I is increased in the Mt-bGH mice with general overexpression of GH, but not in GFAP-bGH mice with overexpression of GH only in the brain (6, 32). Furthermore, circulating IGF-I can reach the brain. Endothelial cells lining brain microvessels express IGF receptors that may internalize IGFs, and infused 125I-labeled IGF-I rapidly crosses the blood-brain barrier (28). IGF-I affects the function of brain dopamine neurons (17), and pharmacological intracerebroventricular administration of IGF ameliorates age-related behavioral deficits (21).

The development of a mouse model with liver-specific, inducible inactivation of the IGF-I gene, using the Cre/loxP conditional knockout system (LI-IGF-I/−/− mice) (29–31, 33), has made it possible to investigate the physiological role of liver-derived IGF-I in adult mice. These mice have increased GH secretion secondary to the decrease in serum IGF-I level [geometric mean plasma GH is 3.1 times higher in LI-IGF-I/−/− mice than in control mice (31, 35)]. The LI-IGF-I/−/− mice do not have decreased IGF-I mRNA expression in the brain (31, 35). Thus this mouse model with low serum levels of IGF-I but high GH levels is well suited for the investigation of whether the effects of GH on activity level and behavior are mediated via a regulation of systemic IGF-I production. In the present study, we determined the role of liver-derived, endocrine IGF-I for locomotion and behavior in adult mice.

MATERIALS AND METHODS

Animals. The LI-IGF-I/−/− mice were created using the Cre/loxP conditional knockout system (18, 20, 29–31, 33). The different genotypes of mice were identified by PCR analysis of DNA from tail biopsies obtained 3 wk after birth. Mice homozygous for loxP and heterozygous for Mx-Cre were given polyinosinic-polycytidylic acid
(PiPc, 6.25 μg/g body wt; Sigma-Aldrich, Stockholm, Sweden) in three intraperitoneal injections at 6 wk (mice aged 6 or 14 mo at encounter with the activity boxes) or 10 wk (mice aged 20 mo at encounter with the activity boxes) to induce expression of Cre protein (18). This results in a specific and complete inactivation of IGF-I in hepatocytes (31). PiPc-treated littersmates, homozygous for loxP but lacking Mx-Cre, were used as controls. One week after the first PiPc injection, blood was collected, and serum was assayed for IGF-I using a commercially available double-antibody, IGF-binding protein-blocked RIA kit (Mediagnost, Tubingen, Germany). In addition, in the 20-mo-old mice, 1 wk after the experiments using the activity boxes were completed, serum IGF-I (Mediagnost), as well as free 3,5,3′-triiodothyronine (T3) and free thyroxine (T4) (RIA, commercial Amerlex-MAB kits; Trinity Biotech, Wicklow, Ireland) were analyzed. The animals had free access to fresh water and food pellets (B&K Universal, Sollentuna, Sweden). The present research was approved by the Ethics Committee at the University of Göteborg.

Activity boxes. Activity level was measured using photocell detection in eight identical Plexiglas boxes (410 × 410 × 200 mm), which were placed in sound-attenuating enclosures with dim lighting (Kungsbacka mät-och Reglerteknik, Fjärrås, Sweden). The activity meter was equipped with 3 × 3 rows of photocell beams, and detectors were placed 100 mm apart at the floor level of the box and at a higher level (30 mm above the floor level), allowing a computer-based system to register the animal’s horizontal and vertical activity, respectively. Activity was defined as interruption of a photocell beam. The software allowed a detailed analysis (8 variables, see Experimental design (activity boxes)) of the activity recorded.

Experimental design (activity boxes). Six-month-old (control: n = 20, 12 females and 8 males; Li-IGF-I−/− mice: n = 20, 12 females and 8 males), 14-mo-old control (n = 11, only male mice) and Li-IGF-I−/− mice (n = 15, only male mice), and 20-mo-old control (n = 17, 10 females and 7 males) and Li-IGF-I−/− mice (n = 20, 15 females and 5 males) were placed in the activity boxes for 60 min (day 1). The experiments were performed between 0900 and 1600 in a quiet environment. The pattern in control animals is shown.

Activity tests in 14-mo-old mice. As shown in Fig. 1, horizontal activity (one count = one breaking of a new beam compared with the previous beam that was broken), horizontal activity (one count = one breaking of one beam in the lower row), peripheral activity (one count = one breaking of one outer edge beam in the lower row), corner time (time during which two outer edge beams were broken at the same time), rearing activity (one count = one breaking of one beam in the upper row), peripheral rearing, and rearing time. Rearing activity therefore reflects the vertical activity of the mice.

Montgomery’s elevated plus-maze. This test, performed 2 wk after the activity tests, consisted of a plus-shaped maze with a mesh wire floor, elevated ~0.75 m above the ground in a semi-illuminated room. The arms of the plus-maze were 40 cm long and 10 cm wide. Two opposing arms were surrounded by black 10-cm-high walls (closed arms), and the other arms were devoid of walls (open arms). Animals were allowed 1 h of habituation to the testing room before the start of the experiment. Each animal was put into an unfamiliar environment, a dark box with a grid floor, for 5 min, after which it was placed in the center of the plus-maze facing a closed arm. Entry into one arm was defined as the animal placing all four paws into the arm. The investigator was situated 2 m from the center of the maze. After every tested animal, the maze was carefully wiped with a wet cloth. Total number of entries, number of entries into an open or closed arm, and time spent in open or closed arms were recorded during the 5-min test sessions.
Activity tests in 20-mo-old mice. In 20-mo-old LI-IGF-I/−/− mice, as measured using the activity boxes, there was a more marked decrease in locomotor activity, horizontal activity, and rearing activity than in the 14-mo-old LI-IGF-I/−/− mice (Fig. 1). In addition, peripheral activity (P < 0.05), peripheral rearing (P < 0.05), and rearing time (P < 0.01) were lower in the LI-IGF-I/−/− than in the control animals, whereas corner time, calculated as the mean AUC value, was similar in both groups (P = 0.13; data not shown).

Locomotor activity 0–60 min (group effect) was similar in the 20-mo-old LI-IGF-I/−/− and the control mice during all trials (Fig. 2). Subanalyses of 20-min intervals for group effect, however, showed lower locomotor activity in the LI-IGF-I/−/− mice during 0–20 min at day 1 (P < 0.05). The time pattern of locomotor activity differed only during day 1, with locomotor activity decreasing less with time in the LI-IGF-I/−/− mice (interaction effect P < 0.05; Fig. 2). Horizontal activity was lower in the 20-mo-old LI-IGF-I/−/− than in the control mice during all trials, and the time pattern differed during days 1 and 3 (Fig. 3). Subanalyses of group effect showed lower horizontal activity in the LI-IGF-I/−/− mice during the first 20 min at day 1 (P < 0.01), during 0–20 min (P < 0.01) and 20–40 min (P < 0.05) at day 3, and during 0–20 and 20–40 min at day 5 (both P < 0.05).

Peripheral activity 0–60 min (group effect) was similar in both study groups during all trials (Table 1). However, subanalyses of 20-min intervals for group effect showed lower peripheral activity during 0–20 min in 20-mo-old LI-IGF-I/−/− mice at day 1 (P < 0.05, Table 1). Peripheral activity decreased less with time in the LI-IGF-I/−/− mice during day 1 (interaction effect P < 0.01; Table 1). Corner time was similar in both groups, and there was no time effect (Table 1). There was, however, a nonsignificant tendency toward a higher corner time in the LI-IGF-I/−/− mice during the trials at day 5 (Table 1).

Rearing activity (0–60 min) was similar in the 20-mo-old LI-IGF-I/−/− and the control mice at day 1 (Fig. 4). At days 3

At days 3 and 5, horizontal activity, rearing activity, peripheral rearing, and rearing time were lower during 0–60 min in the LI-IGF-I/−/− than in the control mice (group effect P < 0.05 for all these variables; data not shown). Subanalyses of 20-min intervals for group effect showed that the most marked differences between the two study groups in horizontal activity and rearing activity were during 0–20 min (data not shown). At day 3, horizontal activity, rearing activity, peripheral rearing, and rearing time decreased less with time in the LI-IGF-I/−/− mice (interaction effect P < 0.05 for all these variables), whereas at day 5 the time pattern was similar in both groups (data not shown).
and 5, rearing activity was lower in the LI-IGF-1/− mice (Fig. 4). This was due to lower rearing activity during 0–20, 20–40, and 40–60 min in the LI-IGF-1/− mice at day 3 (group effect \( P < 0.01 \), \( P < 0.001 \), and \( P < 0.05 \), respectively) and at day 5 (group effect \( P < 0.01 \), \( P < 0.05 \), and \( P < 0.05 \), respectively). The time pattern of rearing activity differed between the study groups at day 3 (interaction effect \( P < 0.05 \)) but not at days 1 or 5 (Fig. 4). Peripheral rearing level and rearing time (0–60 min) were similar in both groups at day 1, whereas at days 3 and 5, peripheral rearing level and rearing time were lower in the 20-mo-old LI-IGF-1/− mice (Table 2).

Analysis of covariance. All significant differences between the 20-mo-old LI-IGF-1/− mice and the control mice also remained significant when sex was used as covariant.

DISCUSSION

This study shows that liver-derived, circulating IGF-I is of importance for exploratory activity in old mice. The LI-IGF-

Montgomery’s elevated plus-maze. In all age groups, there was no difference in any variable measured using Montgomery’s elevated plus-maze. In the 20-mo-old mice, total entries made into both open and closed arms [LI-IGF-1/− mice 11.4 (0.9), controls 12.6 (0.8), \( P = 0.3 \)], number of entries into an open arm (%total entries: LI-IGF-1/− mice 43 ± 3, controls 42 ± 4%, \( P = 0.8 \)) and time spent in open arms (%total time spent in an open arm: LI-IGF-1/− mice 38 ± 5, controls 37 ± 5%, \( P = 0.9 \)) were similar in both groups.

Table 1. Peripheral activity and corner time in 20-mo-old control (n = 17) and LI-IGF-1/− mice (n = 20) at encounter with activity boxes at day 1, day 3, and day 5 (C). Vertical bars indicate SE for mean values shown. Statistics are based on 2-factor repeated-measures ANOVA.

<table>
<thead>
<tr>
<th>Time interval</th>
<th>0–20 min</th>
<th>20–40 min</th>
<th>40–60 min</th>
<th>0–60 min</th>
<th>0–60 min</th>
<th>0–60 min</th>
<th>0–60 min</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral activity (counts/5 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>89.0 ± 15.9</td>
<td>42.4 ± 7.4</td>
<td>23.7 ± 4.2</td>
<td>51.7 ± 8.4</td>
<td></td>
<td></td>
<td></td>
<td>0.26 (&lt;0.001)</td>
</tr>
<tr>
<td>LI-IGF-1/−</td>
<td>55.2 ± 5.5</td>
<td>37.2 ± 7.8</td>
<td>27.8 ± 7.2</td>
<td>40.0 ± 6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>62.1 ± 47.4</td>
<td>38.6 ± 5.9</td>
<td>17.8 ± 5.1</td>
<td>39.5 ± 5.2</td>
<td></td>
<td></td>
<td></td>
<td>0.20 (&lt;0.001)</td>
</tr>
<tr>
<td>LI-IGF-1/−</td>
<td>47.4 ± 6.3</td>
<td>25.1 ± 4.9</td>
<td>19.8 ± 4.3</td>
<td>30.8 ± 4.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>67.1 ± 9.7</td>
<td>33.6 ± 5.5</td>
<td>48.5 ± 23.2</td>
<td>49.7 ± 9.9</td>
<td></td>
<td></td>
<td></td>
<td>0.15 (&lt;0.001)</td>
</tr>
<tr>
<td>LI-IGF-1/−</td>
<td>50.5 ± 7.4</td>
<td>28.0 ± 9.2</td>
<td>20.2 ± 7.1</td>
<td>32.9 ± 6.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corner time (sec/5 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.9 ± 4.4</td>
<td>21.8 ± 11.3</td>
<td>21.9 ± 14.7</td>
<td>19.5 ± 9.0</td>
<td></td>
<td></td>
<td></td>
<td>0.28 0.40</td>
</tr>
<tr>
<td>LI-IGF-1/−</td>
<td>33.4 ± 11.7</td>
<td>40.5 ± 17.1</td>
<td>48.0 ± 20.9</td>
<td>40.6 ± 15.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.4 ± 7.5</td>
<td>45.9 ± 18.3</td>
<td>71.2 ± 30.4</td>
<td>45.8 ± 17.8</td>
<td></td>
<td></td>
<td></td>
<td>0.55 (&lt;0.001)</td>
</tr>
<tr>
<td>LI-IGF-1/−</td>
<td>29.9 ± 13.4</td>
<td>65.8 ± 21.1</td>
<td>88.5 ± 27.6</td>
<td>61.4 ± 18.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.2 ± 10.6</td>
<td>36.1 ± 19.7</td>
<td>43.5 ± 21.4</td>
<td>33.3 ± 16.4</td>
<td></td>
<td></td>
<td></td>
<td>0.09 (&lt;0.001)</td>
</tr>
<tr>
<td>LI-IGF-1/−</td>
<td>60.2 ± 18.7</td>
<td>87.8 ± 23.6</td>
<td>107.2 ± 29.7</td>
<td>85.1 ± 23.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are given as means ± SE. Statistics are based on 2-way repeated-measures ANOVA. In order to have comparable values in all tables and all figures, all values are presented as counts or seconds during 5 min. LI-IGF-1/−, liver-derived IGF-I inactivated. *P < 0.05 vs. control for the given 20-min time interval (2-factor repeated-measures ANOVA, group effect).
IGF-I\(^{-/}\) mice have low circulating IGF-I and compensatory high GH secretion. Six-month-old LI-IGF-I\(^{-/}\) mice had similar activity levels, 14-mo-old LI-IGF-I\(^{-/}\) mice had a moderate but significant decrease in activity level, and 20-mo-old LI-IGF-I\(^{-/}\) mice had a more marked decrease in activity level compared with control mice. There were several signs of an altered habituation process with different time patterns of locomotor activity and horizontal activity both in the 14-mo-old and in the 20-mo-old I-IGF-I\(^{-/}\) mice compared with the age-matched control mice at day 1. At repeated measurements (days 3 and 5), rearing activity was decreased in the 14-mo-old and in the 20-mo-old LI-IGF-I\(^{-/}\) mice.

In this study, in line with previous studies (30), mean body weight was 11–18% lower in the LI-IGF-I\(^{-/}\) than in the control mice. This difference tended to be significant in the 20-mo-old mice and was significant in the 6-mo-old and 14-mo-old mice. Furthermore, longitudinal growth is similar in young LI-IGF-I\(^{-/}\) and control mice (30, 31), whereas in 1-yr-old animals, there are small differences in bone lengths (femur, tibial, and crown rump length: 3.3, 2.1, and 6.6%, respectively, lower in LI-IGF-I\(^{-/}\) mice) (29). These differences in body size are likely not of importance for our results, since there is no correlation between body size and locomotor activity when the activity boxes are used in control mice (weight range 25–45 g) (22). Finally, in the 20-mo-old mice, 75% of the LI-IGF-I\(^{-/}\) and 58% of the control mice were females. This was also not of importance, since all differences also remained when sex was used as a covariant in the statistical analysis.

Table 2. Peripheral rearing and total rearing time in 20-mo-old control (n = 17) and LI-IGF-I\(^{-/}\) mice (n = 20) at their encounter with the activity boxes at days 1, 3, and 5

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral rearing (counts/5 min)</td>
<td>Group</td>
<td>Time</td>
<td>Interaction</td>
</tr>
<tr>
<td>Day 1</td>
<td>Control</td>
<td>20.4 ± 3.9</td>
<td>12.2 ± 2.1</td>
</tr>
<tr>
<td>LI-IGF-I(^{-/})</td>
<td>16.8 ± 3.8</td>
<td>12.8 ± 3.4</td>
<td>9.2 ± 2.9</td>
</tr>
<tr>
<td>Day 3</td>
<td>Control</td>
<td>27.5 ± 5.8</td>
<td>27.3 ± 7.0</td>
</tr>
<tr>
<td>LI-IGF-I(^{-/})</td>
<td>11.0 ± 1.9</td>
<td>7.6 ± 1.7</td>
<td>5.3 ± 1.6</td>
</tr>
<tr>
<td>Day 5</td>
<td>Control</td>
<td>20.1 ± 3.9</td>
<td>12.8 ± 3.6</td>
</tr>
<tr>
<td>LI-IGF-I(^{-/})</td>
<td>11.6 ± 2.1</td>
<td>7.0 ± 2.0</td>
<td>4.0 ± 1.2</td>
</tr>
<tr>
<td>Rearing time (sec/5 min)</td>
<td>Group</td>
<td>Time</td>
<td>Interaction</td>
</tr>
<tr>
<td>Day 1</td>
<td>Control</td>
<td>26.0 ± 5.6</td>
<td>17.2 ± 4.3</td>
</tr>
<tr>
<td>LI-IGF-I(^{-/})</td>
<td>17.2 ± 4.1</td>
<td>14.9 ± 3.8</td>
<td>11.5 ± 3.5</td>
</tr>
<tr>
<td>Day 3</td>
<td>Control</td>
<td>32.8 ± 5.8</td>
<td>34.1 ± 8.2</td>
</tr>
<tr>
<td>LI-IGF-I(^{-/})</td>
<td>12.8 ± 2.5</td>
<td>8.8 ± 2.1</td>
<td>5.7 ± 1.7</td>
</tr>
<tr>
<td>Day 5</td>
<td>Control</td>
<td>31.5 ± 6.1</td>
<td>28.8 ± 7.8</td>
</tr>
<tr>
<td>LI-IGF-I(^{-/})</td>
<td>12.3 ± 2.8</td>
<td>7.9 ± 2.4</td>
<td>5.8 ± 2.0</td>
</tr>
</tbody>
</table>

All values are given as means ± SE. Statistics are based on 2-way repeated-measures ANOVA. In order to have comparable values in all tables and all figures, all values are presented as counts or seconds during 5 min. LI-IGF-I\(^{-/}\), liver-derived IGF-I inactivated, *P < 0.05; **P < 0.01 vs. control for the given 20-min time interval (2-factor repeated measures ANOVA, group effect).
IGF-I is of importance for pre- and postnatal development of the brain. Global inactivation of IGF-I or its receptor results in decreased brain size and number of neurons (12, 34). Furthermore, defects of IGF-I or IGF-I receptors due to gene mutations may be associated with some mental retardation in humans (1, 37). To determine the role of endocrine IGF-I in adult/aging mice, we developed a mouse model with inducible inactivation of liver expression of IGF-I (31). These mice do not have decreased IGF-I mRNA expression in the brain (31, 35). The mice used in the present study were inactivated at 6–10 wk of age, resulting in a complete adult inactivation of IGF-I in hepatocytes, associated with a pronounced reduction in serum IGF-I that was still present at 20 mo of age. Therefore, we can exclude the possibility that the observed alterations in this study were due to developmental changes in the brain or to lack of local IGF-I expression in the brain.

The LI-IGF-I−/− mice had reduced serum IGF-I concentrations (80–85% of controls), and compensatory high GH concentrations [3.1 times higher geometric mean plasma GH level in LI-IGF-I−/− than in control mice (31, 35)]. The Mt-bGH mice, with general overexpression of GH, had a disturbed habituation process with an increased spontaneous locomotor activity throughout 60-min testing at their first encounter with the activity boxes (6, 32). This phenotype is opposite to the phenotype seen in the old LI-IGF-I−/− mice in the present study, and in the 6-mo-old mice the activity level was similar in the LI-IGF-I−/− to that in the control mice despite the compensatory increase in GH secretion in the LI-IGF-I−/− mice. Moreover, in the GFAP-bGH mice, with overexpression of GH only in the central nervous system, there was no increase in spontaneous locomotor activity (6), which indicates that the effects of GH on locomotor activity were mediated by a peripheral factor (6). Therefore, the results of the present study suggest that circulating IGF-I could be a factor that at least partly mediates the effects of GH on locomotor activity.

Horizontal activity (0–60 min) was lower in the 14-mo-old and in the 20-mo-old LI-IGF-I−/− mice than in the controls. At the end of the 60-min periods, however, there was no longer any difference, or only a minor difference, in horizontal activity between the two study groups. This suggests that the decreased horizontal activity was mostly a behavioral response to the encounter with the activity boxes in the LI-IGF-I−/− mice, whereas there was no major difference in basal horizontal activity between the LI-IGF-I−/− and the control mice.

There were several signs of an altered habituation process in the 14-mo-old and in the 20-mo-old LI-IGF-I−/− mice at the first encounter with the activity boxes at day 1. This was most marked in the 20-mo-old LI-IGF-I−/− mice, in which the pattern (interaction effect) of locomotion, horizontal activity, and peripheral activity differed vs. the controls at day 1. Furthermore, the LI-IGF-I−/− and control mice reacted differently to repeated testing. At day 1, rearing activity (0–60 min) was similar in the 14-mo-old and the 20-mo-old LI-IGF-I−/− mice compared with the age-matched controls, whereas at days 3 and 5, rearing activity was lower in the LI-IGF-I−/− mice.

There was no significant difference in any age group in corner time, which could reflect anxiety, fear, or inactivation. There was, however, a nonsignificant tendency toward higher corner time in the 20-mo-old LI-IGF-I−/− mice (group effect P = 0.09 at the third encounter with the activity boxes at day 5). These results suggest that there was no major difference in anxiety level or fear between the LI-IGF-I−/− and control mice. This notion was confirmed by tests using the Montgomery's elevated plus-maze, which did not show any difference between the LI-IGF-I−/− mice and the control mice. The present results, with a decrease in horizontal and rearing activity that was most predominant during the first half of the test periods, combined with unchanged anxiety level, suggest decreased exploratory activity in the LI-IGF-I−/− mice compared with the controls.

The phenotype observed in the LI-IGF-I−/− mice, encounter with the activity boxes, included decreased exploratory activity and an altered habituation process. These disturbances in mice indicate a loss of normal exploratory drive, and possibly energy, and are reminiscent of the mood disturbances observed in GH-deficient adult humans with hypopituitarism and low serum IGF-I concentrations. These GH-deficient patients have decreased well-being, psychic energy and drive, initiative, and ability to cope with stressful situations (2, 5, 8, 11, 23). Because the LI-IGF-I−/− mice have compensatory high GH secretion, the present results might suggest that the mood disturbances in GH-deficient adults are at least partly mediated by low circulating IGF-I concentrations and not by the low GH levels as such. Consequently, it could be hypothesized that the improvement in well-being observed during GH replacement therapy is not a direct effect of GH, but rather mediated via the resulting increase in serum IGF-I levels.

Six-month-old LI-IGF-I−/− mice had similar activity level, 14-mo-old mice had a moderate but significant decrease in activity level, and 20-mo-old LI-IGF-I−/− mice had a more marked decrease in activity level compared with age-matched control mice. Serum IGF-I level decreases in parallel to many of the age-related changes that are observed in normal elderly humans (19), and the present results therefore provide some support for the notion that the age-related decline in serum IGF-I concentration in humans is of importance for age-related changes in activity level and curiosity/exploratory behavior. There are also indications that elderly adult humans with GH deficiency and low serum IGF-I values have more impaired quality of life than young adults with GH deficiency (3, 4, 36). It is, however, unknown whether the regulation by circulating IGF-I on activity level involves neurological or hormonal pathways.

Enhanced spontaneous locomotor activity in the Mt-bGH mice, with general overexpression of GH, is associated with increased corticosterone levels (32). Corticosterone can influence brain dopaminergic systems (5a, 27), which are of importance for spontaneous locomotor activity (14). However, in the GFAP-bGH mice, with overexpression of GH only in the central nervous system (6), serum levels of corticosterone are increased, whereas both circulating IGF-I concentration and spontaneous locomotor activity are similar to those in controls (6). In young LI-IGF-I−/− mice, corticosterone values are increased, whereas in 13-mo-old LI-IGF-I−/− mice corticosterone values are similar to those in controls (30). This argues against corticosterone being involved in the regulation of locomotor activity in these mice strains.

Thyroid hormones have also been suggested to be involved in the GH-induced increase in spontaneous locomotor activity (6, 13, 32). Mt-bGH mice have increased levels of free T3 due to increased conversion of free T4 to free T3 (6, 32), whereas GFAP-bGH mice have unchanged free T3 levels and un-
changed conversion of free T₄ to free T₃ (6). In the present study, free T₄ and free T₃ levels were similar in the 20-mo-old LI-IGF-I⁻/⁻ mice with secondarily increased GH secretion to that in the control mice, suggesting that GH cannot increase free T₄-to-free T₃ conversion without IGF-I. Furthermore, it suggests that the changes in activity level in Mt-bGH, GFAP⁻/⁻, and LI-IGF-I⁻/⁻ mice are at least partly due to changes in circulating IGF-I.

In conclusion, old LI-IGF-I⁻/⁻ mice with low serum IGF-I concentrations and compensatory high GH secretion displayed decreased activity level and an altered habituation process. These findings could suggest that circulating IGF-I mediates, at least partly, the effects of GH on exploratory activity in mice. Furthermore, circulating, liver-derived IGF-I may mediate the beneficial effects by GH on well-being and quality of life in GH-deficient humans.

ACKNOWLEDGMENTS

We are grateful to Kenn Johansson, Maud Petersson, and Anette Hanselvi for excellent technical assistance. We also thank SWEGENE Center for Bio-Imaging, University of Göteborg, for technical support.

GRANTS

This work was supported by grants from the Swedish Medical Research Council (Grant nos. 4247, 254190139, and 11583), the Swedish Foundation for Strategic Research, the Lundberg Foundation, the Torsten and Ragnar Söderberg’s Foundation, the Emil and Vera Cornell Foundation, the Petrus and Augusta Hedlunds Foundation, and the Novo Nordisk Foundation.

REFERENCES

2. Åström C and Lindholm J. Growth hormone-deficient young adults have decreased deep sleep. Neuroendocrinology 51: 82–84, 1990.


