The current studies were initiated to investigate the role of brain-gut peptide, gastrin, on locomotor activity and anxiety-like behavior. Young, male mutant mice, lacking gastrin gene expression (GAS-KO mice), were used in the experiments. The locomotor activity of GAS-KO vs wild type (WT) mice was compared by open field test. The anxiety-like behavior was examined using elevated plus maze. The time and entries to the open arms of the elevated plus maze were used as an indicator for the anxiety-like behavior and the data were analyzed using Hindsight program. On the open field test, locomotor activity of GAS-KO mice was similar to that of the WT mice for the first 10 min of the test, but decreased significantly after that. Anxiety-like behavior was more evident in the GAS-KO vs WT mice in the elevated plus maze experiments. The number of entries to and time spent on the open arms of plus-maze were significantly reduced for the GAS-KO vs WT mice suggesting an increased anxiety-like behavior of GAS-KO mice. Our studies suggest that normal circulating levels of gastrins may play a direct or indirect role in the regulation of locomotor activity and anxiety-like behavior.

Key words: Gastrin, GAS-KO mice, locomotor activity, anxiety-like behavior, elevated plus-maze

INTRODUCTION

We recently reported that gastrin gene knockout (GAS-KO) mice are at high risk for colorectal carcinogenesis compared to the wild type (WT) littermates (1). During the course of our studies, we observed that the GAS-KO mice were becoming increasingly obese and appeared to be more docile and lethargic
compared to their wild type (WT) controls. In further studies, we confirmed that the abdominal adiposity of GAS-KO mice was significantly increased compared to that of WT mice, even at the young age of 2 months old, and that the GAS-KO mice were grossly obese by 6 months of age (2). Since both obesity and reduced physical activity are risk factors for colon carcinogenesis (3, 4), we examined the locomotor activity of GAS-KO versus WT mice in an open field test, in the current studies. Using this method, we confirmed that the locomotor activity of GAS-KO mice was significantly reduced compared to that of the WT mice, at all ages examined. In order to examine if the increased obesity in GAS-KO mice directly co-related with reduced locomotor activity of these mice, we examined the locomotor activity of a sub-set of GAS-KO and WT mice that were either grossly obese or remained lean on a high fat diet. Surprisingly, the locomotor activity of both the obese and lean GAS-KO mice remained significantly lower compared to the locomotor activities of the corresponding WT mice in an open field test.

In order to examine if an increase in anxiety-like behavior may have contributed to the reduced locomotor activity of GAS-KO versus WT mice, we additionally scored the locomotor activity of the mice in an elevated plus maze, by our published methods (5). The time and entries to open and close arms of the maze were used as indicator of anxiety-like behavior and analyzed using Hindsight program (5).

Our results suggest that loss of gastrin gene expression in GAS-KO mice has significant effects on locomotor and anxiety-like behavior of the mice especially in the SV-129 background. Ethical Committee of University UTMB Galveston, USA approved the protocol study.

MATERIAL AND METHODS

Activity measurements

GAS-KO mice were generated and confirmed as reported recently (1). At 2 months of age, the spontaneous exploratory behavior of GAS-KO and WT mice was compared, by utilizing a Photobeam Activity System (PAS) with Flexfield (San Diego Instruments, Inc, San Diego, CA) coupled to a Compaq 486 computer. Activity measurements with age and weight matched mice were monitored and compared, as described previously (6). Briefly, the activity chamber (40 cm x 40 cm x 40 cm) records the number of times the mouse crosses a photobeam grid oriented on an x, y, and z axis. Data for the amount of active and rest time (sec.) and the distance traveled (ft) was collected in 5-minute intervals for 45 min. Rest time was defined as remaining stationary for 1 sec. or longer. Cavicide was used to clean the activity chambers between each experiment, in order to eliminate urine, or other olfactory cues from previous test subjects.

In order to compare the activity of weight matched obese or lean WT and GAS-KO mice, a high fat and high sugar Surwit diet (Research Diets, New Brunswick, NJ) was provided ad libitum, beginning at 6 weeks of age. By 6 months of age, obesity was present in the majority of GAS-KO mice and a few WT mice, providing the opportunity to examine obese and lean subgroups. The
mean weight of the GAS-KO and WT mice on the Surwit diet was determined and the difference between these weights was calculated. A mouse was considered "lean" if it was less than the difference between the GAS-KO and WT mice, and was considered "obese" if it was more than the difference. The weights of the lean mice range from 21-25 gms, while the weight of obese mice ranged from 30-38 gms. Spontaneous exploratory activity measurements were determined for the lean and obese subgroups of GAS-KO and WT mice.

**Elevated Plus-Maze Studies**

The elevated plus-maze experiments were conducted as previously described (5). The apparatus (San Diego Instruments, San Diego, CA) comprised two open arms (30 x 5 cm) and two closed arms (30 x 5 x 15 cm) that extended from a common central platform (5 x 5 cm). A small raised lip (0.5 cm) around the edges of the open arms helped to prevent mice from slipping off. The apparatus was constructed from polypropylene and Plexiglas (white floor, clear walls) and elevated to a height of 38 cm above floor level. Mice were placed on the center square, facing an open arm, and allowed to freely explore the apparatus under even overhead fluorescent lighting (~200 lux) for 5 min. Mice were tested in an order pseudorandomly counterbalanced for genotype, gender and genetic background. Standard behaviors measured were open and closed arm entries (entry = all 4 paws in an arm) and time spent in the open arms. Behavior was scored by an experienced observer, using behavioral scoring software (Hindsight, Scientific Programming Services, Wokingham, U.K.).

**Statistical Analysis**

All values shown in Figs 1 and 2 represent the mean ± SEM. Locomotor data from the brightly lit open field was analyzed using the student's t-test, wherein the data was compared between GAS-KO and WT mice. SigmaStat Software was used for assigning statistical significance. A P value of <0.05 was considered statistically significant. Behavioral data from the elevated plus-maze test were analyzed using analysis of variance (ANOVA) and Newman-Keuls post-hoc comparisons, using stat view (SAS Institute, Inc., Cary, NC). Once again statistical significance was set at p<0.05.

**RESULTS**

**Spontaneous Exploratory Activity**

Mice were placed in an activity chamber to evaluate locomotor activity. Two-month-old weight-matched GAS-KO and WT littermates were monitored simultaneously using the PAS apparatus to electronically monitor the spontaneous exploratory behavior of the mice. As shown in Fig. 1A, during a 45 min period of observation, GAS-KO mice were at rest 47% of the time, while WT mice were at rest only 35% of the time. Thus, there was a 12% increase in the amount of time that GAS-KO mice were stationary, compared to their WT littermates. The activity was also recorded as the distance traveled (ft) during a 45 minute interval (Fig. 1B). GAS-KO mice traveled a distance that was 25% less than that by the WT mice in the chambers (619 ft vs 466 ft; P= <.03). This difference in activity measured in young, weight matched mice indicates that locomotor activity was decreased in the GAS-KO mice at an early age.
Further activity studies were conducted on GAS-KO and WT mice fed a high fat and high sugar diet, in order to promote obesity in WT mice for a weight-matched comparison with obese mice. After 5 months on the Surwit diet, mice were subdivided into lean and obese subgroups, as described in Methods. Fig. 1C compares the average distance traveled (ft/45 min) by the lean and obese subgroups of GAS-KO and WT mice. The distance traveled by the GAS-KO mice was significantly reduced (P<.001) compared to that traveled by the WT mice, in
both the lean (360.8 ± 24.2 vs 607.8 ± 36.4) and obese (311.6 ± 38.6 vs 495.5 ± 27.7) subgroups. Locomotor activity was also graphed as a function of time, as shown in Figs 1D and 1E for the obese and lean subgroups of mice, respectively. The distance traveled by both obese (Fig. 1D) and lean (Fig. 1E) GAS-KO mice was initially similar to that of the WT mice, but decreased with time. Thus, the initial motivation to explore the environment was similar in the GAS-KO and WT mice, but the extent of locomotor activity was decreased after 10 minutes of lateral movement.

Elevated Plus-Maze

The movement of the GAS-KO and WT mice on the open versus closed arms of the elevated plus-maze was scored as described in Methods. GAS-KO mice that were either in the black C57 or the SV-129 backgrounds were examined. The total number of entries (into both the open and closed arms) were similar for the GAS-KO (Black) (14.8 ± 2.4) and WT (Black) (16.1 ± 1.1) mice (data not shown). The total entrances by the GAS-KO (SV-129) mice (5.8 ± 1.2), on the other hand, were significantly lower than that of the GAS-KO (Black) and WT (Black) mice (data not shown). These results suggest that while the initial exploratory behavior of the GAS-KO (Black) and WT (Black) mice was similar, as observed in the open field test (Fig. 1), the exploratory behavior of GAS-KO (SV-129) mice was significantly reduced even in the initial time period; these results suggest that the SV-129 genetic background contributes towards exaggerated behavioral effects that are not seen in the C57 Black background.

The total number of open entrances and the total duration during which the GAS-KO mice (irrespective of genetic background) stayed on the open arm during the 5 minutes of observation, were significantly reduced compared to corresponding values for the WT (Black) mice (Figs 2A and B). The values for open entrances and open duration for GAS-KO (SV-129) mice, on the other hand, were significantly lower than that for GAS-KO (Black) mice (Figs 2A and B). Unlike the open arm values, the total duration for which the mice stayed on the closed arms of the elevated plus-maze were similar for all three groups of mice as shown in Fig. 2C. The total number of entrances to the closed arms were similar for the GAS-KO (Black) (9.19 ± 1.8) and WT (Black) (9.1 ± 0.8) mice (data not shown). The total number of entrances by the GAS-KO (SV-129) mice to the closed arms, however, were significantly lower (3.9 ± 0.8) (data not shown), confirming that the ambulatory activity of GAS-KO mice in the SV-129 background was significantly reduced.

Since only the number of open entrances and duration (time spent) on the open arms were significantly reduced for GAS-KO (Black) versus WT littermates, it suggests that the anxiety-like behavior of GAS-KO (Black) mice is significantly enhanced compared to that of WT (Black) littermates. In the case of GAS-KO (SV-129) mice, since all three parameters of total, open and closed entrances
were significantly reduced, it suggests that not only the anxiety-like behavior was elevated in these mice but also the ambulatory and exploratory behavior was significantly reduced.

DISCUSSION

In the current studies we examined if a possible loss in locomotor activity of the GAS-KO vs WT mice may explain the recently observed increase in the obesity of GAS-KO mice (2). In the first set of experiments, spontaneous exploratory behavior of the mice was examined in an open field test. Our results suggest that while the initial exploratory behavior was not changed, the locomotor activity of 2 month old GAS-KO mice, over a period of time, was significantly decreased compared to that of the WT mice. Thus, a decrease in locomotor activity from an early age may have contributed to the increase in obesity in GAS-
KO mice, just as sedentary lifestyles can lead to obesity and type II diabetes in humans (3, 4).

In a second set of experiments, mice were placed on a high fat and high sugar diet in order to accelerate the onset of obesity and to allow a comparison between diet-induced obese WT and GAS-KO mice. The WT mice and GAS-KO mice were then divided into subgroups of lean vs obese mice, and the locomotor activity of the mice examined. GAS-KO mice, irrespective of their body weights, demonstrated a significant decrease in locomotor activity compared to weight matched WT mice (Fig. 1). Thus, GAS-KO mice demonstrated reduced locomotor activity compared to the WT littermates, irrespective of weight changes. Interestingly, our experiments revealed that the initial motivation to explore the environment was similar in the GAS-KO and WT mice. However, after 10-15 min, locomotor activity of the GAS-KO mice subsided significantly, suggesting that either anxiety and/or muscular fatigue may have contributed to the lethargic phenotype of the GAS-KO mice. In preliminary studies we have compared the ATP levels in the skeletal muscles (from the thighs) of the mice, and so far have not measured any significant differences in the ATP levels in the skeletal muscles of GAS-KO vs WT mice at the ages of 2-4 months of age. It thus appears likely that factors other than ATP levels in the skeletal mass and muscular fatigue may have contributed to the reduced physical activity of GAS-KO vs WT mice even at very young ages.

Our results with the elevated plus maze suggest that the anxiety-like behavior of GAS-KO mice was significantly increased compared to that of the WT mice, which may have contributed to the reduced locomotor activity of these mice in an open field test. The genetic backgrounds of the GAS-KO mice were found to exaggerate the relative levels of anxiety-like behavior in the GAS-KO mice. The GAS-KO mice in the SV-129 background demonstrated a 4-5 fold increase in anxiety-like behavior compared to GAS-KO mice in the C57 black background. It appears likely that both the genetic background and the loss of gastrin gene expression, impact the phenotype of the mice (such as obesity, locomotor activity and anxiety-like behavior).

The mechanisms mediating the significant changes in the phenotype of the GAS-KO versus WT mice, are as yet unknown, but may include a differential expression of the CCK2 receptor (R) subtypes in the various parts of the brain. Many studies have reported an important and significant role of CCK1-R and CCK2-R (that are mainly expressed in the brain and gut tissues of rodents and humans, 7, 8), on various locomotor and behavioral patterns (9 - 22). CCK receptors are distributed in several brain regions, such as amygdala, hypothalamus and cortex, which control anxiety behaviors (7, 8). In addition, CCK2-R co-localize with dopamine receptors in the striatum and ventrotagmental area, which control locomotor activity (reviewed in 9), anxiety-like behavior and panicogenic effects in response to endogenous, locally available CCK in the brain, has been demonstrated (reviewed in 7 - 9). Thus CCK in the brain regulates anxiogenic and panicogenic behaviors (7 - 9, 12). On the other hand, CCK2-R knockout mice were
reported to demonstrate increased anxiety-like behaviors (11, 15, 19). The reasons why loss of CCK2-R, on the one hand, and administration of CCK agonists, on the other hand, result in similar anxiogenic effects is not understood.

The current studies, for the first time, suggest that loss of circulating amidated gastrins in GAS-KO mice, also results in a similar anxiety-like behavior on the elevated plus-maze as has been reported for CCK2-R knock out mice (11).

In previous studies it has been reported that amidated gastrins are required for up-regulation of CCK2-R in the stomach (reviewed in 23). In the absence of gastrin gene expression (as in GAS-KO mice), CCK2-R expressing cells (such as parietal cells in the stomach) are significantly reduced (discussed in 1). It is thus possible that a loss in gastrin gene expression may similarly result in down regulation of CCK2-R expressing neurons in the brain, which may explain a similar phenotype of GAS-KO and CCK2-R-KO mice. Loss in CCK2-R binding in the brain was reported to increase anxiogenic effects in rats (14), giving further credulence to this possibility. It is thus possible that pathways mediating anxiogenic effects in GAS-KO mice may be similar to the pathways mediating similar effects in the CCK2R-KO mice. Studies are planned to examine this intriguing possibility.

Based on the results so far, it is possible that the observed increase in obesity in the GAS-KO mice may be related to the reduced locomotor activity and the increased anxiety in these mice. Peripheral CCK is believed to play an important role in mediating satiety effects *via* CCK1-R (24-27), and CCK1-R agonists are being developed to treat obesity (24). Surprisingly, food consumption by the GAS-KO mice was similar to that of the WT mice (2) suggesting that satiety does not play a role in the observed increases in the obesity of GAS-KO mice.

Gastrins mediate their biological effects not only *via* CCK2-R, but also *via* several other receptor subtypes including CCKC-R and the more recently described progastrin receptors (PG-R) (23, 28 - 30). At the present time it is not known if these newly described receptor subtypes are expressed in the brain. It is possible that besides direct/indirect effects of gastrins *via* CCK-2R (as described above), gastrins may also have direct effects on behavioral patterns by interacting with other receptor subtypes, such as PG-R, in the brain. This possibility will be examined in future studies.

**Acknowledgements:** This work was supported by NIH R01-CA72992, NIH R01-CA60087, and NIH R01-CA97959 to PS and by departmental funds to SC. The technical help of Owlia, A. is acknowledged and the secretarial support of Lindie Nanninga for typing the manuscript is greatly appreciated.

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Received: November 15, 2003
Accepted: January 15, 2004

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