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ORIGINAL ARTICLE

Stimulation of defecation in spinal cord-injured rats by a centrally acting ghrelin receptor agonist

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Study design: Animal proof of principle study.
Objectives: To determine whether capromorelin, a compound that causes defecation by stimulating ghrelin receptors within the lumbosacral defecation centers, is effective after spinal cord injury (SCI), and whether SCI significantly alters sensitivity to the compound.
Setting: University of Melbourne and Austin Hospital, Melbourne, Australia.
Methods: Rats were subjected to spinal cord contusion injury or were sham-operated. At 6 weeks after surgery, effects of capromorelin on blood pressure, heart rate and propulsive contractions of the colorectum were investigated.
Results: Capromorelin caused robust propulsive activity in the colorectum soon after its application. The compound was similarly effective in naïve, sham-operated and spinal cord-injured rats. Blood pressure increases caused by capromorelin were not exaggerated after SCI, and there was no evidence of phasic blood pressure increases when the colon was contracted by the compound.
Conclusion: Capromorelin is a therapeutic compound that could potentially be used to relieve constipation by triggering defecation in spinal cord-injured patients.
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Keywords: bowel dysfunction; spinal cord injury; defecation; ghrelin receptors

Introduction

A major problem identified in spinal cord injury (SCI), affecting about 40% of patients, is an inability to empty the bowel when defecation is convenient and a leakage of bowel contents at inappropriate times. In many patients this is the most distressing aspect of SCI.[1] Failure of normal neural control of the bowel can have significant side effects, including impaction, hemorrhoids, rectal bleeding, prolapse, formation of anal fissures and chronic constipation, leading to megacolon requiring operative diversion.[2] The most common approaches to bowel management are manual emptying and use of laxatives.

Another possible approach would be activation of the defecation centers in the lower spinal cord. Most SCIs are at cervical and thoracic levels, whereas the defecation centers are located at lumbosacral levels. It has been recently discovered that centrally penetrant compounds that are agonists of the ghrelin receptor act in the lumbosacral spinal cord to stimulate defecation.[3] Defecation can be initiated by either intravenous or oral application of ghrelin receptor agonists that cross into the central nervous system.[3,4] The effects of peripheral administration are mimicked by direct application of ghrelin itself or of ghrelin receptor agonists to the spinal cord.[3,5] The effects on the colorectum are prevented by cutting the neural connections between the spinal cord and the large intestine, but are not affected by acutely severing the spinal cord rostral to the lumbosacral region.[3]

The characteristics of the lumbosacral micturition centers, that are also spared by cervical or thoracic cord section, are changed following spinal injury at more rostral sites.[6] This generally results in an initial areflexia, which later changes to a hyper-reflexia and involves enhanced reflex activity through the micturition center.

The first purpose of this study was to determine whether activation of the lumbosacral defecation center with a ghrelin receptor agonist was effective in causing defecation in rats that had a previous severe injury to the thoracic cord. The second purpose was to investigate whether adaptation to the injury resulted in a changed sensitivity of the defecation center. We have used capromorelin because it is orally active, penetrates into the central nervous system, potent at ghrelin receptors and active and safe in human.[7]

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Materials and methods

The experiments used male Sprague–Dawley rats, and procedures were approved by the University of Melbourne Animal Experimentation Ethics Committee.

Spinal cord lesion

A total of 15 rats received a contusion injury at T10. The rats were injured at 4–10 weeks of age and left for 6 weeks to recover (experimented at 10–16 weeks). A total of 17 sham operations were conducted at the same ages. Spinal contusion lesions were made using aseptic conditions under inhaled isoflurane anesthesia (3% in oxygen). The muscles connecting to the spines of the vertebrae were detached and a laminectomy was performed to remove the dorsal aspect of the T10 vertebra. Animals were transferred to a spinal stereotaxic frame and the vertebral column was secured. A contusion injury was made at T10 using a computer controlled impactor device[8] with the depth of penetration of the impactor tip set at 2 mm, the impact velocity at $1\,m\,s^{-1}$ and the dwell time of the tip in the spinal cord at 100 ms. The laminectomy was closed with three layers of sutures through the adjacent vertebral musculature, the subdermal tissues and the skin. The area was then disinfected with chlorhexidine in 70% alcohol. Sterile saline ($5\,ml\,kg^{-1}$) and analgesic, buprenorphine ($0.06\,mg\,kg^{-1}$), were administered postoperatively by intraperitoneal injection. After recovery from anesthesia, the animals were examined to confirm paralysis of the hind limbs as an index of the completeness of the lesion. Additional injections of sterile saline and analgesic were administered once daily for the first 4 days postoperatively. The rats were fed with normal rat chow for the 6 weeks after surgery. The food was placed in containers on the cage floor in the first 2 weeks to allow easy access. For the first 5–7 days, all rats had distended bladders that were expressed manually by gentle squeezing, while the rats were under light isoflurane anesthesia. The extent of the spinal cord lesion was checked histologically after the completion of the physiological studies.[8]

Physiological studies

Rats were sedated with ketamine hydrochloride ($50–60\,mg\,kg^{-1}$, intramuscular) and anesthesia was induced with α-chloralose ($60\,mg\,kg^{-1}$, intravenous). The femoral artery was cannulated for the infusion of anesthetic and blood pressure recording, and the femoral vein was cannulated for drug delivery. Blood pressure and heart rate were recorded with a Power Lab recording system using Chart 5 software (both from ADInstruments, Sydney, Australia). Anesthesia was maintained by intra-arterial infusion of α-chloralose ($12–20\,mg\,kg^{-1}\,hr^{-1}$) plus ketamine ($3–5\,mg\,kg^{-1}\,hr^{-1}$) in phosphate buffered saline. Colonic motility was recorded as described previously.[3] The distal colon was cannulated at the colonic flexure, which in the rat is at the junction of the proximal and distal colon, where formed fecal pellets are first observed. A second cannula was placed at the anus. The muscle and skin were closed around the proximal cannula. The oral cannula was connected to a Marriotte bottle filled with warm phosphate buffered saline, and the distal cannula to a pressure transducer via a one-way valve. The baseline intralumenal pressure was maintained at 3–5 mm Hg by adjusting the heights of the Marriotte bottle and outlet. Expelled fluid was collected in a cylinder distal to the one-way valve, and measured by weighing with a force transducer. Blood pressure measurements were made continuously and pressures were averaged over 20 min periods for analysis. Capromorelin ($4\,mg\,kg^{-1}$) was injected via the femoral vein. At the end of each experiment, the rat was killed with a lethal dose of sodium pentobarbitone ($300\,mg\,kg^{-1}$ intravenous), while still under anesthesia.

Data analysis

Analyses were performed using Graph Pad Prism (Graph Pad Software Inc., San Diego, CA, USA). Where the variances of the groups were similar, the data are presented as means and s.e.m.'s, and comparisons were made using one way analysis of variance followed by *post-hoc* analysis using the Tukey–Kramer method for multiple group comparisons. Where the variances of the groups differed significantly (assessed by Levene's test), the data are presented as medians and interquartile ranges, and comparisons were made by a Kruskal–Wallis test followed by pairwise comparisons using the Mann–Whitney *U*-test. The level of significance was set at $P<0.05$.

Reagents

The following were used: capromorelin (CP424391; Pfizer Pharmaceuticals, Sandwich, UK); sodium pentobarbitone (Sigma Aldrich, Sydney, Australia); buprenorphine (Reckitt Benckiser, Sydney, Australia); and 2-hydroxypropyl-β-cyclodextrin (Wacker-Chemie GmbH, Burghausen, Germany). Capromorelin was dissolved in 15% w/v β-cyclodextrin.

Results

From the total of 47 rats used, 15 were naïve controls, 17 were sham-operated and 15 underwent SCI surgery.

Histological analysis revealed destruction of greater than 80% of the cross-sectional area of the spinal cord at the center of the lesion in SCI animals, with only a thin rim of residual white matter remaining underneath the pial surface (Figure 1). As with other studies in rats, bladder function was initially lost but was restored within the first 2 weeks after the injury. The rats initially dragged their hind limbs. Improvement in hind limb function was apparent by 2 weeks and rats were weight bearing on their hind limbs by 2–4 weeks, although there was no evidence of supra-spinal control. In our previous studies using the same protocol for producing SCI, the performance of rats on the ledged beam test and the random rung ladder test (both assessing hind limb function) showed that hind limb function was still deficient at 6 weeks.[8] There was no loss of spinal tissue or function in the sham-operated rats.

Physiological measurements before drug administration

The mean resting arterial blood pressure under α-chloralose plus ketamine anesthesia averaged about 75 mm Hg in both

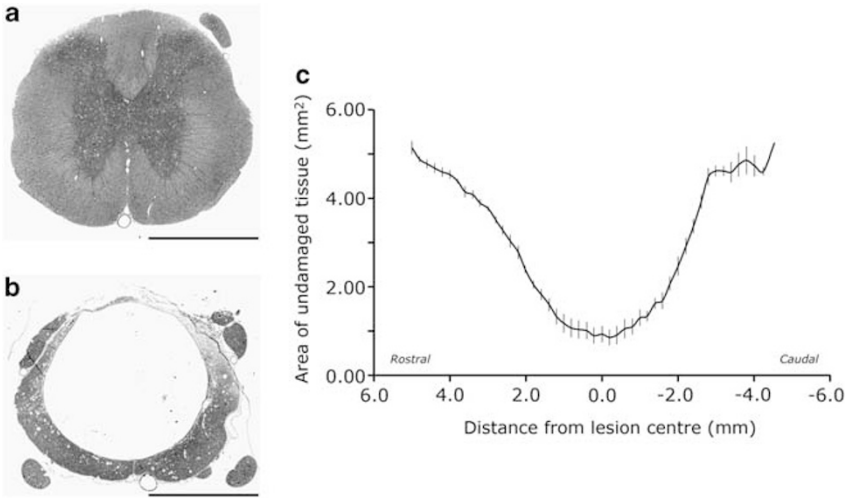

Figure 1 Effects of the contusion injury on the spinal cord. Images are of resin embedded 0.5 μm thick sections from normal spinal cord (**a**) and from a region of the cord 1 mm caudal to the center of the contusion, 6 weeks after injury (**b**). The majority of the tissue is lost after SCI, with only a peripheral rim being retained. (**c**) Quantitative morphometric analysis of tissue loss after 6 weeks, mean cross-sectional areas ± s.e.m., measured at 200 μm intervals, $n=6$. There was substantial tissue loss up to 2 mm rostral and caudal to the center of the impact, which was made with an impactor with a flat tip of 2 mm diameter. Scale bars on A and B are 1 mm.

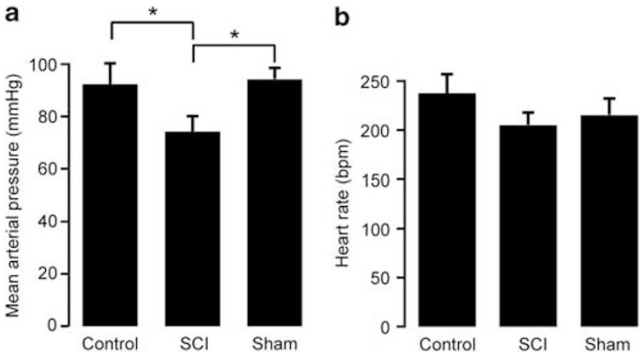

Figure 2 Blood pressures (**a**) and heart rates (**b**) in rats at 10–16 weeks of age, which in the case of spinal cord-injured (SCI) animals and sham-operated animals were 6 weeks after surgery. Data are mean arterial pressure and heart rate during anesthesia before the administration of capromorelin, averaged over 20 min. The blood pressure was significantly lower in SCI rats compared with control (naïve, unoperated) and sham-operated rats (*$P<0.05$), but control and sham-operated animals were not different. Heart rates were not significantly different between groups ($P<0.05$). Data, mean ± s.e.m.

control, unoperated rats and sham-operated rats, 6 weeks after surgery (Figure 2). Mean resting arterial blood pressure was about 20 mm Hg lower at 6 weeks after SCI, significantly different from sham or unoperated rats. The resting heart rates were not significantly different between groups.

Responses to capromorelin

Capromorelin (4 mg kg^{-1}, intravenous) increased colorectal activity and blood pressure in rats from all three groups, naïve control, sham-operated and SCI animals (Figures 3–5). A series of phasic increases in colorectal pressure was observed in naïve control, sham-operated and SCI rats that commenced within 20–60 s after drug administration. The phasic increases in colorectal pressure were often associated with increases in expulsion of fluid through the anal cannula (Figure 3), but were not associated with phasic changes in blood pressure. The colorectal pressure increases were not always tightly related to fluid expulsion, especially when the contractions were closely spaced. Lack of correlation may be cased by inertia in the recording system and by contractions occurring in different regions of the colorectum. Following capromorelin administration, the blood pressure increased over a period of 1–2 min (Figure 3), remained at about the same level for 10–25 min and then declined over the next 10–20 min to its pre-drug level. The blood pressure increases did not differ between the naïve control, sham-operated and SCI groups, either in the peak blood pressure response or in the average increase in the 20 min following drug delivery (Figure 4). The number of propulsive contractions of the colorectum during the 20 min following capromorelin administration did not differ significantly between the groups of animals, and the duration of the response to this agent in SCI rats did not differ from that in naïve control or sham-operated rats (Figure 5). The duration of the response to capromorelin was, however, significantly briefer in sham-operated rats than in naïve control rats.

Discussion

These studies show that the stimulant of defecation, capromorelin, a selective agonist for ghrelin receptors, has similar effects in SCI rats, naïve or sham-operated rats. This and other ghrelin receptor agonists that cross the blood–brain barrier, and ghrelin applied directly to the spinal cord, stimulate autonomic preganglionic neurons that express the ghrelin receptor.[3,5,9]

SCI at cervical or thoracic levels disrupts descending inputs that control the lumbosacral defecation centers.

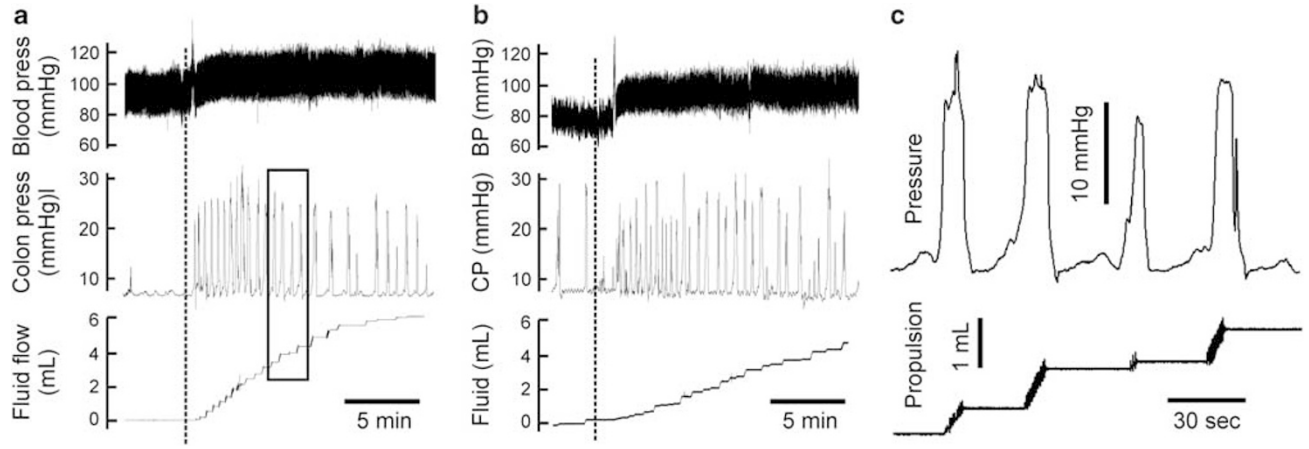

Figure 3 Individual records of blood pressure (BP) and colorectal motility changes in response to capromorelin in a naïve control (unoperated) rat (**a**) and in a spinal cord-injured rat (**b**). In both cases, capromorelin (4 mg kg^{-1}, intravenous, at the dashed lines) caused an increase in blood pressure (upper traces), phasic propulsive pressure waves in the colorectum (middle traces) and propulsion of the fluid content from the colorectum (lower traces). The segments of colon pressure and fluid flow traces in the box in **a** are enlarged in **c**. This indicates that the phasic increases in colonic pressure are propulsive contractions that can often be temporally linked to fluid propulsion from the colon.

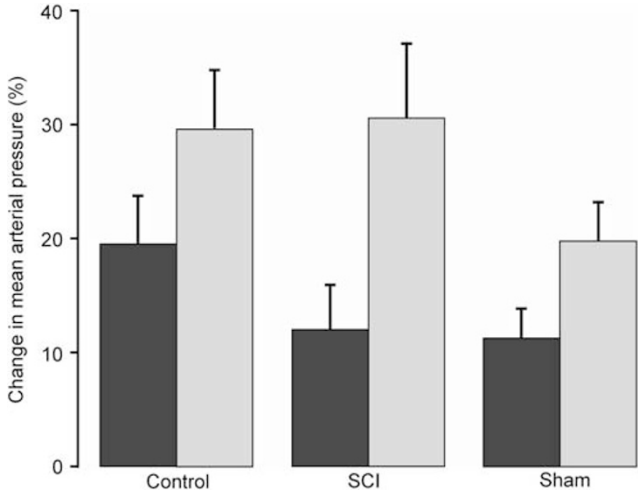

Figure 4 Average (black) and peak (gray) blood pressure increases in response to capromorelin (4 mg kg^{-1}, intravenous) in control (naïve, unoperated), sham-operated and spinal cord-injured (SCI) rats. Blood pressure was averaged over 20 min before drug application and for the first 20 min after administration of capromorelin. Capromorelin increased blood pressure by 10–20 mm Hg, and the increase did not differ significantly between groups. Likewise, there were no differences in the peaks of the blood pressure responses. Data, mean ± s.e.m.

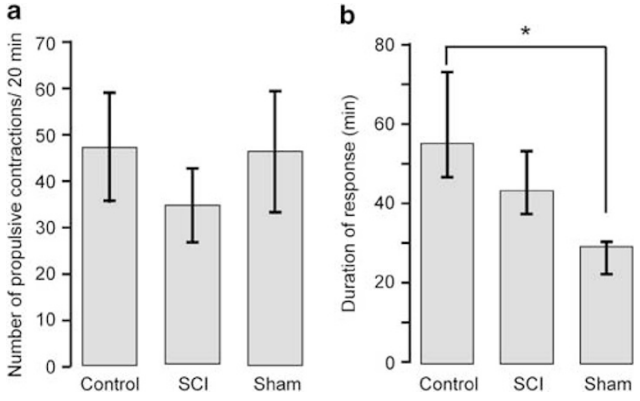

Figure 5 Numbers of colonic contractions in the 20 min following capromorelin (**a**) and the durations (**b**) of responses to capromorelin (4 mg kg^{-1}, intravenous) in control (naïve, unoperated), sham-operated and spinal cord-injured rats, 6 weeks after surgery. There were 0–5 contractions per 20 min before the addition of capromorelin. Responses were similar in the naïve and SCI groups. In comparison with the control rats, but not the SCI rats, the duration of response in sham-operated rats was reduced (*$P<0.05$). Data are mean ± s.e.m. for the propulsive contractions and median and extents of second and third quartiles for the durations, for which the data were skewed.

Disruption of inputs to neurons in the central nervous system can cause receptor supersensitivity,[10] as is well documented for peripheral neuro-effector systems. In SCI, receptor expression and responsiveness are changed in the spinal cord below the level of the lesion[11,12] and there is reorganization of nerve circuits.[13] Hyperresponsiveness occurs in peripheral tissues whose neural control is disrupted by SCI.[14] Thus, the responsiveness of the defecation centers to ghrelin receptor stimulants might be changed after SCI. However, we found that capromorelin was effective in stimulating colorectal propulsive activity after SCI at the same dose as in controls. The neural pathway from the spinal defecation centers to the muscle of the colon involves neuro-neuronal connections in the enteric nervous system (within the bowel wall) and transmission from both enteric excitatory and enteric inhibitory neurons to the muscle. Thus, although there may be changes at different points in the reflex pathway, the overall consequence for the action of ghrelin agonists is that their effectiveness is unchanged.

The significant reduction in the duration of the response of the colorectum in sham-operated rats is interesting. The sham operation causes some inflammation at the site of operation, but the white matter tracts passing from the

region of operation to the lumbosacral regions are not severed. It is possible that inflammation causes changes at more caudal sites that are lost when pathways are interrupted by the spinal cord contusion injury. Consistent with the current observations, in a previous study we found that responses of arteries to nerve stimulation were reduced in sham-operated animals 7 weeks postoperatively, a change that may also be explained by inflammation at the laminectomy site.[15]

After SCI, resting blood pressure is decreased,[16] as we confirmed in the present study. However, as some pathways were spared by the contusion injury used in the present study, our results do not provide information on the extent that blood pressure would be affected by a complete transection at T10. Although blood pressure is lower in SCI, it is also less stable and disturbances to viscera can elicit dysreflexic episodes, characterized by sudden large blood pressure increases.[16] We thus need to consider whether the compounds such as capromorelin that both increase blood pressure and stimulate visceral organs, including the colon, might cause exaggerated increases in blood pressure. In fact, we found that both the peak blood pressure responses and the increases in mean resting arterial blood pressure over a 20 min period after drug application were not different in SCI animals compared with either naïve or sham-operated controls. Thus, the current experiments give no support to the hypothesis that blood pressure responses to ghrelin receptor agonists would be exaggerated. Moreover, the contractions of the colon induced by capromorelin were not accompanied by phasic blood pressure rises. Unlike the dysreflexic blood pressure increases that occur when the colon is distended in spinal cord-injured rats,[17] the events in the colon and the blood pressure changes that are observed in response to ghrelin agonists in rats without SCI are independent.[5,9] When they are applied at sites rostral to the defecation centers in normal animals (and rostral to the site of SCI used in the current experiments), ghrelin receptor agonists only elicit blood pressure responses (maximal blood pressure effects are from T9 to T12) and only small blood pressure increases are elicited from the levels of the defecation centers at L1 to L3.[9] Moreover, the effects on the colon can be blocked pharmacologically without affecting the blood pressure increases that occur when ghrelin receptors are activated.[5]

It remains possible that if the SCI had been above T5, a level that in rats severs the bulbospinal pathways controlling the splanchnic circulation, hyper-reflexic blood pressure responses would have been elicited. We have been unable to locate any published data that indicate that centrally penetrant ghrelin receptor agonists increase blood pressure in human. In fact, it appears that the peripheral vasodilator effects of ghrelin agonists, which have been well-documented in animal studies,[9] dominate in humans.[18] Ghrelin itself, which does not cross into the spinal cord, also lowers blood pressure in human.[19]

Thus, the present study predicts that the ghrelin receptor agonist, capromorelin, or ghrelin receptor agonists with similar pharmacokinetic profiles, could be given to human SCI patients in doses similar to those utilized in other studies.[7] Although trials of ghrelin receptor agonists have been conducted for other indications, it has been found that the agonists increase defecation in human.[20] Thus, we conclude that centrally penetrant ghrelin receptor agonists may have a therapeutic role in causing defecation in spinal cord-injured patients who are unable to initiate defecation themselves. However, whether capromorelin would be suitable following SCI above the T6 level cannot be predicted by this study.

Conclusions

This study provides animal proof of principle that a ghrelin receptor agonist that can cross the blood–spinal cord barrier can cause defecation after SCI with a similar potency as in normal animals. This opens the way for further investigations that could lead to a useful therapy for problems of bowel function that are common in spinal cord-injured individuals.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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