J Mol Neurosci (2012) 48:558–564
DOI 10.1007/s12031-012-9839-9

Intranasal Application of Secretin, Similarly to Intracerebroventricular Administration, Influences the Motor Behavior of Mice Probably Through Specific Receptors

Andrea Heinzlmann · Gusztáv Kiss · Zsuzsanna E. Tóth · Roberta Dochnal · Ágnes Pál · Ildikó Sipos · Máté Manczinger · Gyula Szabó · Hitoshi Hashimoto · Katalin Köves

Received: 27 March 2012 / Accepted: 11 June 2012 / Published online: 1 July 2012

Abstract Secretin and its receptors show wide distribution in the central nervous system. It was demonstrated previously that intravenous (i.v.) and intracerebroventricular (i.c.v.) application of secretin influenced the behavior of rat, mouse, and human. In our previous experiment, we used a special animal model, Japanese waltzing mice (JWM). These animals run around without stopping (the ambulation distance is very limited) and they do not bother with their environment. The i.c.v. secretin attenuated this hyperactive repetitive movement. In the present work, the effect of i.c.v. and intranasal (i.n.) application of secretin was compared. We have also looked for the presence of secretin receptors in the brain structures related to motor functions. Two micrograms of i.c.v. secretin improved the horizontal movement of JWM, enhancing the ambulation distance. It was nearly threefold higher in treated than in control animals. The i.n. application of secretin to the left nostril once or twice a day or once for 3 days more effectively enhanced the ambulation distance than i.c.v. administration. When secretin was given twice a day for 3 days it had no effect. Secretin did not improve the explorative behavior (the rearing), of JWM. With the use of in situ hybridization, we have found very dense secretin receptor labeling in the cerebellum. In the primary motor cortex and in the striatum, only a few labeled cells were seen. It was supposed that secretin exerted its effect through specific receptors, mainly present in the cerebellum.

Keywords Japanese waltzing mouse · Rat · Open field test · Ambulation distance · Rearing

Abbreviations

GABA	γ-Amino butyric acid
i.c.v.	Intracerebroventricular
i.n.	Intranasal
i.v.	Intravenous
JWM	Japanese waltzing mice
LTP	Long-term potentiation
NST	Nucleus of solitary tract
PV	Paraventricular nucleus
SO	Supraoptic nucleus

A. Heinzlmann · K. Köves (✉)
Department of Human Morphology and Developmental Biology,
Faculty of Medicine, Semmelweis University,
Tűzoltó u. 58,
Budapest 1094 Hungary
e-mail: koves.katalin@med.semmelweis-univ.hu

G. Kiss · R. Dochnal · Á. Pál · I. Sipos · M. Manczinger · G. Szabó
Department of Pathophysiology, Faculty of Medicine,
Albert Szent-Györgyi University,
Szeged, Hungary

Z. E. Tóth
Neuromorphological and Neuroendocrine Research Laboratory,
Department of Anatomy, Histology and Embryology,
Semmelweis University and the Hungarian Academy of Sciences,
Budapest, Hungary

H. Hashimoto
Laboratory of Medicinal Pharmacology,
Graduate School of Pharmaceutical Sciences, Osaka University,
Osaka, Japan

Introduction

Secretin in the nervous system was first demonstrated in the forebrain and brain stem using radioimmunoassay and high-pressure liquid chromatography (Charlton et al. 1981). In the rat cerebellar Purkinje cells secretin and its mRNA were first demonstrated by Yung et al. (2001) using in situ hybridization. A few years later, secretin was also demonstrated in human cerebellum (Lee et al. 2005). Later we found secretin immunoreactivity in the Purkinje cells, central cerebellar nuclei, pyramidal cells of cortex, hippocampus, amygdala, and in sensory ganglia of the rat, cat, and human (Köves et al. 2002, 2004). Secretin mRNA was demonstrated in the trigeminal ganglion (Heinzlmann et al. 2011). Two questions arises: what is the function of secretin and where is the site of action of secretin in the central nervous system?

Function of Secretin in the Central Nervous System

At the first time, the effect of secretin on the behavior of rats was also demonstrated by Charlton et al. (1983). Secretin administered intracerebroventricularly (i.c.v.) to rats decreased the novel object approach and the open field locomotor activity, however, did not influence the rearing. In our previous experiment (Köves et al. 2011), we studied the effect of secretin administered i.c.v. on the behavior of two mouse strains. The response to secretin of CFLP white mice and Japanese waltzing mice (JWM) was compared. CFLP mice are usually used for testing the effect of various drugs on the behavior. JWM were included because they exhibit repetitive movement, which is characteristic for several mental disorders, especially for autistic children. These animals run around in their cage nearly without stopping not bothering their environment. Their ambulation distance is much lower than that of CFLP mice. The data showed that secretin influenced the locomotor activity of the above-mentioned special animal model. Its effect on the repetitive movement was beneficial. It enhanced ambulation distance to the level of CFLP mice, although it did not improve the explorative behavior of the animals (rearing). It has also become evident that secretin is synthesized in supraoptic (SO) and paraventricular (PV) nuclei (Welch et al. 2004) and it regulates water homeostasis (Chu et al. 2009). In PV, there may be an interaction between secretin and corticotropic hormone-releasing hormone (Welch and Ruggiero 2005). More than a decade ago, it was found that secretin counteracts the analgesic effect of morphine (Babarczy et al. 1995).

Site of Action of Secretin in the Central Nervous System

The site of action of secretin was investigated to induce c-Fos activation by administration of secretin or to demonstrate secretin receptors. Intravenous (i.v.) infusion of secretin to rats induced c-Fos gene expression in the central amygdala, area postrema, bed nucleus of stria terminalis, external lateral parabrachial, and SO (Goulet et al. 2003). After i.c.v. administration of secretin, many regions such as several brain stem, hypothalamic and limbic structures (septum and amygdala), the medial bank of the anterior prefrontal, orbitofrontal, and the piriform cortex showed activation of Fos protein in awake, freely moving rats (Welch et al. 2003). It is clear from the above-mentioned data that secretin influences c-Fos expression in structures that are involved in behavior, stress adaptation, and visceral responses. There is a limited number of papers in the literature which demonstrated the presence of secretin receptors in the structures where secretin induced c-Fos expression or appearance of Fos protein. Fremeau et al. (1983) using radioreceptor assay found secretin binding in the cerebellum, cortex, thalamus, striatum, hippocampus, hypothalamus, and the brain stem. Nozaki et al. (2002) found in vitro I^{125}-secretin binding in the nucleus of solitary tract (NST), laterodorsal thalamus, accumbens nucleus, hippocampus, cerebellum, cingulate, and orbital cortex. Tay et al. (2004) using polymerase chain reaction found the highest secretin receptor level in the cerebellum, although it was also present in NST, amygdala, hippocampus, and area postrema. Cheng et al. (2011) using in situ hybridization described the presence of secretin receptors in PV and arcuate nucleus. Yung et al. (2001) were able to show secretin receptors in the basket and Purkinje cells of the cerebellar cortex.

Intranasal Application of Peptides

In the last two decades, some peptides such as cholecystokinin (Pietrowsky et al. 1996, 2001; Born et al. 2002; Illum 2004; Schneider et al. 2009), thyreotropic hormone-releasing hormone (Kubek et al. 2009) and insulin (Guthoff et al. 2010) were shown to be delivered to the brain by intranasal (i.n.) application. In a recent work, Nonaka et al. (2009) demonstrated that pituitary adenylate cyclase-activating polypeptide applied i.n. could be delivered to the brain and its distribution might be influenced with coadministration of α- or hydro-β-cyclodextrin. It was also shown that an iodinated secretin analogue could enter the brain from the nose (Banks et al. 2002).

Aim of Experiments

1. The first aim of our present experiment was to elaborate the i.n. application of secretin in JWM mice. The advantage of this method that it is not invasive and it could be easily used not only in adult patients but in children as well who have mental disorders.
2. The second aim was to compare the effect of secretin administered i.c.v. and i.n. Open field test was used to investigate the effect of secretin on the behavior of JWM.

3. The third aim of this experiment was to demonstrate secretin receptors in areas which are related to motor functions. We have used in situ hybridization in rats.

Materials and Methods

Animals Adult (3–4 month old) female JWM were used for the behavior experiments and Sprague-Dawley adult male rats were used to look for secretin receptors. The animals were kept in a light- and temperature-controlled vivarium (lights on at 500 hours and lights off at 1900 hours; temperature 22±2 °C). Treatment of animals was in accordance with the rules of the "European convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986; permission #: 22.1/1158/2010).

Administration of Secretin One hundred micrograms of secretin (Sigma-Aldrich, St. Louis, MO) was dissolved in 5 μl acetic acid+20 μl distilled water. Then to the 25-μl solution, containing 100 μg secretin, 75 μl physiological saline or artificial cerebrospinal fluid was added. The final concentration was 1 μg/μl secretin.

1. Intracerebroventricular application. Under general hexobarbital anesthesia (0.08 mg/gr BW), a guiding cannula was implanted in the lateral ventricle of ten JWM. A day later to five animals, 2 μg secretin in 2 μl artificial cerebrospinal fluid was administered through the cannula. The rest of the animals received only cerebrospinal fluid.
2. Intranasal application through a bougie. The needle of a Hamilton syringe (10 μl) was equipped with a piece of polyethylene tube (length 3.5 mm, inner diameter 0.015 in,, outer diameter 0.043 in.). Ten JWM received 2 μg secretin in 2 μl physiological saline and it was sprayed into the left nostril by the syringe. Five animals received 2 μl physiological saline.
3. Intranasal application in pulverized form. Nine JWM received 2 μg secretin in 2 μl physiological saline in pulverized form once or twice in three succeeding days. Four animals served as controls. According to the similar schedule, they received physiological saline.

The effect of secretin on behavior of the animals was examined using an open field test.

Behavior Test The open field apparatus was a square open field black cage with a side length of 60 cm, surrounded by a 40-cm high wall. The floor of the cage was divided in 36 (6×6) small squares. A 60-W light was situated 1 m above the arena floor. Sessions started at 9 a.m. All animals were carried to the experimental room in their home cage. Each animal was placed in the center of the open field and was observed for 30 min. Conducta 1.0 system (Experimetria Ltd., Budapest, Hungary) was used for monitoring the animals and analyzing the data. The horizontal locomotor activity defined as ambulation distance and the vertical movement defined as rearing were evaluated. Statistical analysis of the data was made by repeated measure analysis of variance (ANOVA). For significant ANOVA values, groups were compared by Tukey's test as post test. A probability value, $p<0.05$ was considered statistically significant.

In Situ Hybridization

1. Five animals were decapitated; the brain was immediately removed and stored at −70 °C until use. Twelve-micrometer thick sections were cut on cryostat (Cryotome, Thermo Shandon, Pittsburg, PA), mounted on silanized slides, and dried on a hot plate (37 °C).
2. The 453-base pair-long fragment (Ishihara et al. 1991) was shortened and subcloned into a pBluescript II SK (+) vector and verified by sequencing. Antisense and sense cRNA probes, labeled by [^{35}S] uracil triphosphate and used for in situ hybridization, were produced by vitro transcription.
3. Before hybridization sections were washed and then treated with 0.25 % acetic anhydride in 0.1 M triethanolamine HCl (pH 8.0) for 10 min. After hybridization, the sections were rinsed in 0.3 M NaCl–0.03 M sodium citrate solution (2× SSC), dehydrated and delipidated in a subsequent series of 70, 85, 95, 100, and 95 % ethanol, and finally air-dried. Hybridization was performed overnight at 55 °C with 10^6 cpm/slide radioactively labeled secretin receptor riboprobe in a humid chamber. The following day, the sections were washed in 4× SSC buffer (pH 7.0) for 4×5 min at room temperature; then they were treated in RnaseA (20 μg/ml; Sigma, Budapest, Hungary) buffer (pH 8.0) containing 500 mM NaCl, 10 mM Tris–HCl, and 0.25 mM ethylenediaminetetraacetic acid for 30 min at 37 °C, then for 5 min in each of the following buffer solutions: 2× SSC, 1× SSC, and 0.5× SSC at room temperature; and finally in 0.1× SSC at 65 °C for 2×30 min. Then the slides were let to cool down and washed in 1× PBS (pH 7.4). The slides were dipped into NTB3 nuclear track emulsion (Eastman Kodak Co., Rochester, N.Y.). After 21 days of exposition time at 4 °C in dark, the reaction was developed using Kodak Dektol developer and fixer at 18 °C.
4. The slides were counterstained with Giemsa solution, dried, and coverslipped with DePeX.

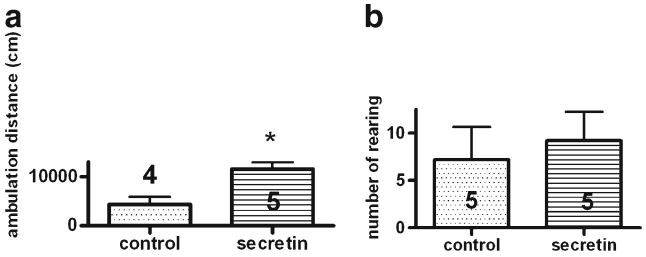

Fig. 1 **a** The diagram shows the quantitative analysis of the horizontal movement of the animals after 2 μg of i.c.v. secretin. Ambulation distance is expressed in centimeters for 30 min. *$p<0.05$. **b** The vertical movement, that is the number of rearing, was not influenced by i.c.v. administration of secretin

Results

Effect of Secretin on the Behavior

The effect of i.c.v. and i.n. application of secretin to the animals was compared.

1. The i.c.v. application of secretin significantly influenced the horizontal movement of the animals. It enhanced the ambulation distance. It was more than twofold higher than in control animals (Fig. 1a). Rearing in untreated JWM is very low, and the animals do not bother about their environment. The i.c.v. application of secretin did not improve this parameter (Fig. 1b).
2. The i.n. application of secretin, sprayed into the left nostril once or twice a day, was even more effective enhancing the ambulation distance in the experimental animals than the i.c.v. application (Fig. 2a), but it was not able to influence the rearing similarly to i.c.v application (Fig. 2b).
3. The i.n. application of secretin in pulverized form through a bougie in the left nostril once or twice for 3 days had contrasting effect. Once a day administration (three times all together) enhanced the ambulation distance in the experimental animals similarly to the single i.n. administration. The ambulation distance was also higher than in controls (Fig. 3a). When secretin was administered twice a day for 3 days, that is, when it was overdosed (six times all together), it had no effect and the ambulation distance was similar to that of the control animals. None of these treatments could influence the rearing (Fig. 3b).

Secretin Receptors in Structures Related to Motor Functions

In the primary motor cortex, we found a few, weekly labeled secretin receptor-expressing cells. They were seen in the median frontal gyrus. In the head of caudate nucleus (striatum), similarly to the frontal gyrus, a few very weekly labeled cells were observed (not shown). However, in the cerebellum, a very dense secretin receptor expression was found. The labeling was denser in the hemispheres (Fig. 4a) than in the vermis (Fig. 4b). As it was expected, many labeled cells were observed in the cortex, but the labeling was not observed in the central cerebellar nuclei. The majority of the labeled cells were found in the granule cell layer. Figure 4c–f shows secretin-expressing cells in this layer. Purkinje cells were rarely labeled (Fig. 4d). Figure 4e clearly shows the place of unlabeled Purkinje cells. By chance we could see the labeled cells in the molecular layer as well (Fig. 4e). In Fig. 4f, very weekly labeled cells are shown in the granule cell layer of the vermis.

Discussion

The regulation of the behavior of animals is a very complex process; many structures of the nervous system are involved in this mechanism. They exert their effect through neuropeptides and neurotransmitters. One of these peptides may be the secretin. The presence of secretin in the central nervous system and the effect of secretin on the behavior became evident in the last three decades as it was described in the "Introduction."

To better understand the neuroactive role of secretin in the brain, Yamagata et al. (2008) have recently generated secretin-deficient mice. Exon 1 of the secretin receptor was replaced with *lacZ* reporter and *PGKneobpA* selection marker. In mutant mice, lacZ reporter was demonstrated in the molecular layer of the hippocampus, dentate gyrus,

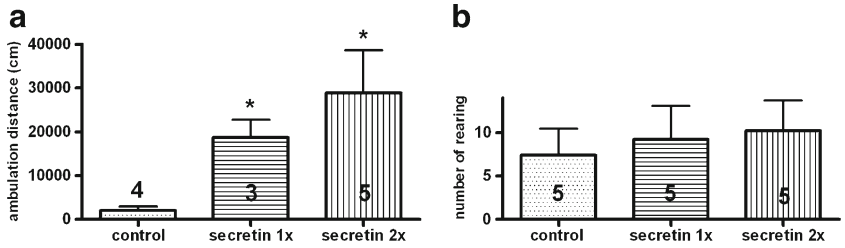

Fig. 2 **a** The diagram shows the quantitative analysis of the horizontal movement of the animals after 2 μg of i.n. administration of secretin through a bougie and is expressed in centimeters for 30 min. Secretin given once or twice a day enhanced the ambulation distance. *$p<0.05$. **b** Number of rearing of the animals was not influenced at all

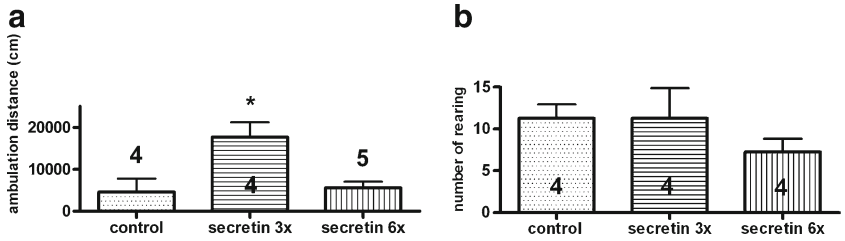

Fig. 3 **a** The diagram shows the quantitative analysis of the horizontal movement of the animals after 2 μg of i.n. administration of secretin in pulverized form once or twice for 3 days (three times or six times). Ambulation distance is expressed in centimeters for 30 min. Secretin given three times enhanced, but given six times did not influence, the ambulation distance. *$p<0.05$. **b** Secretin did not influence the rearing in these animal groups

posterior commissure, habenula, cerebellum, olfactory glomeruli, and the dorsal tegmentum. No obvious morphological and developmental abnormalities were found in the brain of these animals. Electrophysiological examination was only carried out in the hippocampus using electrophysiological approach. A reduction in LTP induction and LTP maintenance was found. These results suggest that both secretin and its receptor are necessary for normal hippocampal function. The effect of secretin is probably mediated through the glutamate, an excitatory amino acid, critical for LTP. This hypothesis is supported by the findings of Kuntz et al. (2004) who demonstrated that secretin increased glutamate and γ-amino butyric acid (GABA) levels in the rat hippocampus using microdialysis technique.

The effect of secretin may be mediated via the structures of the nervous system where secretin receptors are found. It is well known that the cerebellum coordinates the motor activity of the organism. Both secretin and its receptors were demonstrated by Yung et al. (2001, 2006). In the cerebellum, secretin facilitates the GABAergic inhibitory inputs onto Purkinje cells via a postsynaptic- and cAMP-dependent mechanism as a retrograde messenger. The

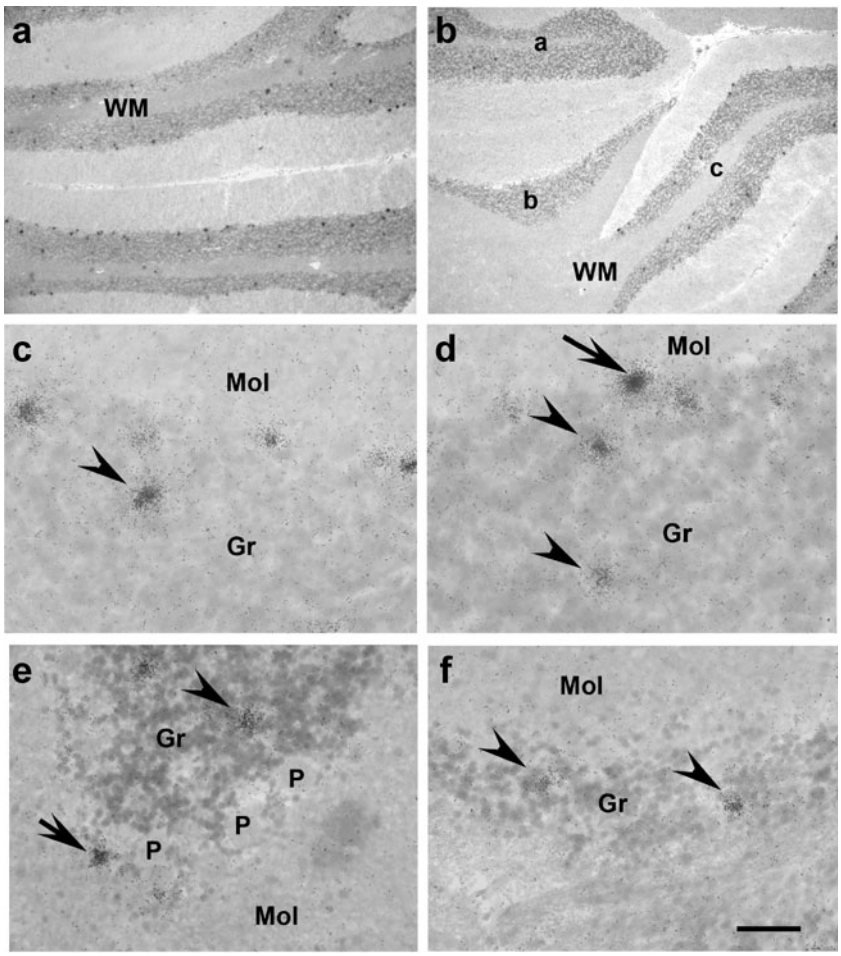

Fig. 4 Microphotographs show secretin-expressing neurons in the cerebellar cortex. **a** Two folia in the left hemisphere. *Black spots* indicate secretin receptor-expressing cells. **b** Two folia in the vermis (*a* and *b*) and one in the right hemisphere (*c*). **c–e** High-power details of the left hemisphere. **f** High-power detail of the vermis. *Arrow* in **d** indicates silver grain containing secretin receptor-expressing Purkinje cell, *arrow* in **e** shows a secretin receptor-expressing cell in the molecular layer, *arrowheads* show labeled cells in the granular cell layer. Abbreviations: *Gr* granular cell layer, *Mol* molecular layer, *P* Purkinje cell, *WM* white matter. Scale, 250 μm in **a** and **b**, and 30 μm in **c–f**

participation of the cerebellum in the stereotypic movements was also suggested. With the use of magnetic resonance imaging, Pierce and Courchesne (2001) observed that the size of the VI and VII lobes of the cerebellar vermis is negatively correlated with the seriousness of the repetitive movements in autistic patients.

In our previous work, we investigated the effect of secretin applied i.c.v. on the locomotor activity and on the explorative behavior in JWM and CFLP white mice. Our animal model, JWM, showed a repetitive circular movement, a stereotypic behavior which also occurs in several mental disorders. Secretin strikingly improved the stereotypy. The animals exhibited more linear movement instead of circular one. In the computerized analysis, it was indicated by the enhancement of the ambulation distance during the observation period. The secretin did not influence the explorative behavior which was indicated by rearing of animals. In our previous experimental conditions (Köves et al. 2011), none of the parameters were influenced by secretin in the control CFLP white mice. In this present experiment, it was found that i.n. application of secretin is even more effective than the i.c.v. application to JWM.

It was supposed that secretin could exert its abovementioned effect through specific receptors. We looked for secretin receptors in brain structures related to motor functions. In the primary motor cortex and the striatum, only a few neurons expressed secretin receptors; however, a dense labeling was seen in the cerebellum. Our probe could mainly detect secretin receptors in the granular cell layer; scattered labeled cells were seen in molecular layer as well. A few Purkinje cells were also labeled. Our results correlate with those described by Yung et al. (2001, 2006). They demonstrated secretin receptor labeling in the Purkinje and basket cells of the cerebellar cortex and also showed that secretin facilitated the GABAergic inhibitory inputs onto Purkinje cells via a postsynaptic- and cAMP-dependent mechanism as a retrograde messenger. A few years earlier, Nishijima et al. (2006) demonstrated that secretin receptor-deficient mice moved significantly faster than wild-type mice. In our special animal model, the pattern of movement was changed by administration of secretin. The untreated rats exhibited circular movement; however, the treated rats mainly exhibited horizontal movement.

We have also seen considerable secretin receptor expression besides the cerebellum in the NST, the laterodorsal thalamic nucleus, and lateral habenula (unpublished data). The latter two structures belong to the limbic system. It is also possible that secretin can influence the other aspects of the behavior of animals, which was not investigated in our work, through these structures. Nishijima et al. (2006) investigated not only the motor functions but among others, the social behavior of secretin receptor mutant mice. They used a tube for social dominance and partition test for social interest and recognition. It was found that the mutant mice exhibited abnormal social behavior.

In summary, our data show that secretin influences the locomotor activity of a special animal model. Its effect on the repetitive movement is beneficial. It normalizes the horizontal movement (ambulation distance), although it does not improve the explorative behavior of the animals. It seems that the i.n. application is more effective than the i.c.v. one; however, its overdosing had no effect. The presence of secretin receptors in the structures related to motor function suggests that the effect of secretin is mediated through these specific receptors.

Acknowledgments This work was partially supported by the Department of Human Morphology and Developmental Biology, Semmelweis University and ETT Grant (355-08) to Gyula Szabó. We say thanks to Anna Takács and Kerti Judit for their technical assistance.

References

Babarczy E, Szabó G, Telegdy G (1995) Effects of secretin on acute and chronic effects of morphine. Pharmacol Biochem Behav 51:469–472

Banks WA, Goulet M, Rusche JR, Niehoff ML, Boismenu R (2002) Differential transport of a secretin analog across the blood–brain and blood–cerebrospinal fluid barriers of the mouse. J Pharmacol Exp Therap 302:1062–1069

Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL (2002) Sniffing neuropeptides: a transport to the human brain. Nat Neurosci 5:514–516

Charlton CG, O'Donohue TL, Miller RL, Jakobowitz DM (1981) Secretin immunoreactivity in rat and pig brain. Peptides 2:45–49

Charlton CG, Miller RL, Crawley JN, Handelmann GE, O'Donohue TL (1983) Secretin modulation of behavioral and physiological functions in the rat. Peptides 4:739–742

Cheng CYY, Chu JYS, Chow BKC (2011) Central and peripheral administration of secretin inhibits food intake in mice through the activation of the melanocortin system. Neuropsychopharmacology 36:459–471

Chu JYS, Lee LT, Lai CH, Vaudry H, Chan YS, Yung WH, Chow BKC (2009) Secretin as a neurohypophyscal factor regulating body water homeostasis. Proc Natl Acad Sci USA 106:15961–15966

Fremeau RT Jr, Jensen RCG, Miller RL, O'Donohue TL, Moody TW (1983) Secretin: specific binding to rat brain membranes. J Neurosci 3:1620–1625

Goulet M, Shiromani PJ, Ware CM, Strong RA, Boismenu R, Rusche A (2003) Secretin i. v. infusion activates gene expression in the central amygdala of rats. Neuroscience 118:881–8

Guthoff M, Grichisch Y, Canova C, Tschritter O, Veit R, Hallschmid M, Häring HU, Preissl H, Hennige AM, Fritsche A (2010) Insulin modulates food-related activity in the central nervous system. J Clin Endocrinol Metab 95:748–755

Heinzlmann A, Tóth ZE, Köves K (2011) Secretin mRNA in the subdivision of primary sensory neurons in the trigeminal ganglion of rats. J Mol Neurosci 43:101–108

Illum L (2004) Is nose-to-brain transport of drugs in man a reality? J Pharm Pharmacol 56:3–17

Ishihara T, Nakamura S, Kaziro Y, Takahashi T, Takahashi K, Nagata S (1991) Molecular cloning and expression of a cDNA encoding the secretin receptor. Embo J 10:1635–1641

Köves K, Kausz M, Reser D, Horváth K (2002) What may be the anatomical basis that secretin can improve the mental functions in autism? Regul Pept 109:167–172

Köves K, Kausz M, Reser D, IIIyés G, Takács J, Heinzlmann A, Gyenge E, Horváth K (2004) Secretin and autism: a basic morphological study about the distribution of secretin in the nervous system. Regul Pept 123:209–216

Köves K, Kiss G, Heinzlmann A, Dochnal R, Manczinger M, Pál Á, Sípos Á, Szabó G (2011) Secretin attenuates the hereditary repetitive movements in a mouse model. J Mol Neurosci 43:109–114

Kubek MJ, Domb AJ, Veronesi MC (2009) Attenuation of kindled seizures by intranasal delivery of neuropeptide-loaded nanoparticles. Neurotherapeutics 6:359–371

Kuntz A, Clement HW, Lehnert W, Van Calker D, Hennighausen K, Gerlach M, Schulz E (2004) Effects of secretin on extracellular amino acid concentrations in rat hippocampus. J Neural Trans 111:931–939

Lee SM, Yung WH, Chen L, Chow BK (2005) Expression and spatial distribution of secretin and secretin receptor in human cerebellum. Neuroreport 16:219–222

Nishijima I, Yamagata T, Spencer CM, Weeber EJ, Alekseyenko O, Sweatt D, Momoi MJ, Ito M, Armstrong DL, Nelson DL, Paylor R, Bradley A (2006) Secretin receptor deficient mice exhibit impaired synaptic plasticity and social behavior. Hum Mol Genet 15:3241–3250

Nonaka N, Nakamachi T, Shioda S, Banks WA (2009) Delivery of pituitary adenylate cyclase activating polypeptide (PACAP) to the brain: targeting with intranasal delivery and cyclodextrins. The 9th International Symposium on VIP, PACAP and Related Peptides, abstract O-9, p. 54.

Nozaki S, Nakata R, Mizuma H, Nishimura N, Watanabe Y, Kohashi R, Watanabe Y (2002) In vitro autoradiographic localization of ^{125}I-secretin receptor binding sites in rat brain. BBRC 292:133–137

Pierce K, Courchesne E (2001) Evidence for a cerebellar role in reduced exploration and stereotyped behavior in autism. Biol Psychiatry 49:655–664

Pietrowsky R, Specht G, Fehm HL, Born J (1996) A nose–brain pathway for psychotropic peptides: evidence from a brain evoked potential study with cholecystokinin. Psychoneuroendocrinology 21:559–572

Pietrowsky R, Claassen L, Frecks H, Fehm HL, Born J (2001) Time course of intranasally administered cholecystokinin-8 on central nervous effects. Neuropsychobiology 43:254–259

Schneider R, Osterburg J, Buchner A, Pietrowsky R (2009) Effect of intranasally administered cholecystokinin on encoding of controlled and automatic memory processes. Psychopharmacology (Berl) 202:559–67

Tay J, Goulet M, Rusche J, Boismenu R (2004) Age related and regional differences in secretin and secretin receptor mRNA levels in the rat brain. Neurosci Lett 366:176–181

Welch MG, Ruggiero DA (2005) Predicted role of secretin and oxytocin in the treatment of behavioral and developmental disorders: implication for autism. Int Rev Neurobiol 71:273–315

Welch MG, Keune JD, Welch-Horan TB, Anwar N, Anwar M, Ruggiero DA (2003) Secretin activates visceral brain regions in the rat including areas abnormal in autism. Cell Mol Neurobiol 23:817–37

Welch MG, Keune JD, Welch-Horan TB, Anwar N, Anwar M, Ludwig RJ, Ruggiero DA (2004) Secretin: hypothalamic distribution and hypothesized neuroregulatory role in autism. Cell Mol Neurobiol 24:219–241

Yamagata T, Urano H, Weeber EJ, Nelson DL, Nishijima I (2008) Impaired hippocampal synaptic function in secretin deficient mice. Neuroscience 154:1417–1422

Yung WH, Leung PS, Ng SSM, Zhang J, Chan SCY, Chow BKC (2001) Secretin facilitates GABA transmission in the cerebellum. J Neurosci 21:7063–7068

Yung WH, Chan YS, Chow BKC, Wang JJ (2006) The role of secretin in the cerebellum. Cerebellum 5:43–48