Upregulation of the Endogenous Peptide Antisecretory Factor Enhances Hippocampal Long-term Potentiation and Promotes Learning in Wistar Rats

Lisa Wintzell, a, Samuel Klemetz, a Stefan Lange, b Eric Hanse, a Caroline Wass c and Joakim Strandberg a,d*,y

a Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden
b Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden
c Department of Pharmacology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden
d Department of Clinical Neurophysiology, Sahlgrenska University Hospital, Göteborg, Sweden

Abstract—Antisecretory Factor (AF) is an endogenous peptide known for its powerful antisecretory and anti-inflammatory properties. We have previously shown that AF also acts as a neuromodulator of GABAergic synaptic transmission in rat hippocampus in a way that results in disinhibition of CA1 pyramidal neurons. Disinhibition is expected to facilitate the induction of long-term potentiation (LTP), and LTP is known to play a crucial role in learning and memory acquisition. In the present study we investigated the effect of AF on LTP in CA3-CA1 synapses in rat hippocampus. In addition, endogenous AF plasma activity was upregulated by feeding the rats with specially processed cereals (SPC) and spatial learning and memory was studied in the Morris Water Maze (MWM). We found that LTP was significantly enhanced in the presence of AF, both when added exogenously in vitro as well as when upregulated endogenously by SPC-feeding. In the presence of the GABA A-receptor antagonist picrotoxin (PTX) there was however no significant enhancement of LTP. Moreover, rats fed with SPC demonstrated enhanced spatial learning and short-term memory, compared with control animals. These results show that the disinhibition of GABAergic transmission in the hippocampus by the endogenous peptide AF enhances LTP as well as spatial learning and memory. © 2022 The Author(s). Published by Elsevier Ltd on behalf of IBRO. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Key words: disinhibition, GABA, hippocampus, memory, Morris water maze.

INTRODUCTION

The Antisecretory Factor (AF) is a 41 kDa endogenous protein which act in vivo by regulating the transport of water and ions across the cellular membrane (Johansson et al., 1995; Lange and Lonnroth, 2001). AF presence has been demonstrated in all mammals’ tissues and body fluids investigated so far (Johansson et al., 2009; Lange et al., 1999; Lange and Lonnroth, 2001). This protein has been ascribed to play a regulative, central role in the immune system due to its presence in the dendritic cells, in B-cells and in macrophages, but also in various forms of lymphoid tissues (Davidson and Hickey, 2004).

In the physiologically, normo-balanced organism most of the AF content is present in an inactive form. AF can, however, be stimulated to activity as well as to increased synthesis by e.g. bacterial toxins (Lange and Lonnroth, 2001), but also after oral intake of hydrothermally processed cereals commonly designated as SPC-Flakes® (specially processed cereals). This endogenous AF stimulation, achieved after intake of the processed cereals, is the result of a balanced content of sugars and amino acids in the feed and has been demonstrated to be beneficial for patients suffering from several inflammatory and/or hypersecretory conditions (Bjorck et al., 2000; Laurenius et al., 2003; Svensson et al., 2004; Finkel et al., 2004; Hanner et al., 2010). SPC-containing feed has also been demonstrated to induce AF in various animals including rats (Johansson et al., 2011).

The detailed AF mechanism of action remains to be described, but the nervous system seems to play a central role in close conjunction with binding proteins and membrane transport channels (Matson Dzebo et al., 2014; Nawrot-Porabka et al., 2015; Nicolas and Lievin-Le Moal, 2015; Bazzurro et al., 2018; Ilkhanizadeh et al., 2018). The conclusive results of AF
action upon the various cellular activities is to bring the different constituent in the single cell as well as in the different tissues into a normo-regulative, physiological balance (Lonnroth et al., 2016; Johansson et al., 2018).

A high concentration of preformed, active AF can be demonstrated in the egg yolk of egg-laying hens fed an SPC-enriched diet (Goransson et al., 1993; Kaya et al., 2017). Oral intake of a spray dried form of this egg-yolk, designated Salovum®, has proven clinically effective in different pathophysiological conditions (Eriksson et al., 2003; Zaman et al., 2018; Gatzinsky et al., 2020). SPC-Flakes® and Salovum® have both been categorized as “Food for specific medical purposes” by the Swedish and EU regulatory, medical authorities. These products have been on the market since 1993, and so far no unwanted, medical side effects has been reported.

The AF protein has been sequenced, and the biologically, active site of the AF protein is located in the amino terminal part of the sequence (Johansson et al., 1997). Due to stability and potency, the amino acid sequence 36–51, designated AF-16, has been thoroughly tested in vivo as well as in vitro. AF-16 has been found capable of mediating anti-secretory as well as anti-inflammatory effects in several different in vivo (Jennische et al., 2008; Al-Oldama et al., 2011; Al-Oldama et al., 2015; Lange et al., 2020) and in vitro (Rapallino et al., 2003) test systems. Conclusively, AF-16 was selected for our in vitro studies.

We have previously shown that AF also have an effect on GABAergic transmission in the rat hippocampus in that it gives a disinhibition of CA1 pyramidal cells (Kim et al., 2005). This disinhibition was mediated by an enhancement of tonic GABAergic inhibition of the CA1 stratum radiatum interneurons (Strandberg et al., 2014). Such a disinhibition would be expected to facilitate the induction of LTP (Wigstrom and Gustafsson, 1983) and possibly learning.

In the present study we investigated how AF affects long-term potentiation (LTP) in CA3-CA1 synapses in the rat hippocampus. Furthermore, since LTP is also involved in learning and memory (Mansvelder et al., 2019; Nicoll, 2017), we also investigated how AF might affect spatial memory and learning. These experiments were performed by testing the rats with the Morris water maze (MWM) test.

**EXPERIMENTAL PROCEDURES**

**Animals**

Male adult (P35-60) Wistar rats bred at the local animal facility (EBM Gothenburg) were used for all experiments. Room temperature and humidity were kept constant at 20 °C and 55%, respectively. Rats were given ad libitum access to food and water. Daylight cycle was maintained artificially, lights off between 18:00 and 07:00 h and the experiments were executed during the light phase. Animals were housed in cages of three to six animals per cage in a colony room. The study was performed according to Swedish legislation on animal welfare with ethics permission given by the Ethics Committee for Animal Experiments, Gothenburg and in accordance with the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Animals used for experiments in MWM were housed for a minimum of 21 days before experiments began in order to acclimatize them to their new environment as well as human handling. There was a significant difference in average weight between the treatment groups used for experiments in the MWM and subset of electrophysiological experiments before feeding with SPC flakes begun (average within the SPC-group = 91.33 g, n = 13, in controls = 108.70, n = 12, p = 0.021), but not on the first acquisition day of the MWM (average weight in the SPC-group = 218.71 g, in controls = 227.11 g, p = 0.39), nor on the day after the MWM was finished (average weight in the SPC group = 245.51 g, in controls = 251.29 g, p = 0.58).

For electrophysiological experiments on hippocampal slices from rats fed with SPC, 4 out of 10 slices in the SPC group and 3 out of 10 slices in the control group were from animals that did not perform the MWM task. There were no significant differences in the amount of LTP between slices from animals that performed MWM compared to slices from animals that did not. Experiments on slices from rats that did perform the MWM task were performed 3–20 days after the MWM task was ended for the SPC group and 4–20 days after the MWM task was ended for the control group.

**AF-16 peptide and induction of endogenous AF activity**

The AF-16 peptide (VCHSK TRSNP ENNVG L) (Johansson et al., 1995; Johansson et al., 1997) was synthesized and chemically characterized to more than 98% purity, by Ross-Petersen AS, Copenhagen, Denmark.

Endogenous AF activity was upregulated by feeding rats with a standard rodent diet containing 5% SPC for at least 21 days before testing in MWM and/or electrophysiological experiments begun. It has previously been readily demonstrated in humans, pigs, chickens, rats and cows that feeding with ordinary, commercially available feed supplemented with SPC in a concentration range of 5–15% and a 18–21 days long feeding period, induce a stimulated, high endogenous AF activity when determined in plasma/milk with ELISA (Lange and Lonnroth, 2001). The researcher was blinded to treatment status of each animal during all experiments and statistical analysis.

**Electrophysiology**

Animals were deeply anaesthetized by isoflurane before decapitated using a guillotine. The brain was quickly removed, the cerebral hemispheres separated and put in an ice-cold (≤3 °C) slicing solution containing (in mM): glycerol 219; KCl 2.5; NaH2PO4 1.2; CaCl2 1.2; MgCl2 7; NaHCO3 26; glucose11 which was saturated with 95% oxygen and 5% carbon dioxide. By using a vibratome (Microm HM 650 V, Thermo Fisher Scientific, Loughborough, UK) the hemispheres were cut into 400 μm thick transverse slices in the same solution.
A cut between the CA1 and CA3 was made to prevent spreading of spontaneous network activity and the hippocampal slices were then transferred to a storage chamber with an artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 123; NaH₂PO₄ 1; NaHCO₃ 26; KCl 3; glucose 10; CaCl₂ 2; MgCl₂ 4, perfused with 95% oxygen and 5% carbon dioxide and holding a temperature of 25 °C (the higher concentration of Mg²⁺ and lower temperature, compared to recording conditions, were used to reduce neuronal activity thus reducing potential excitotoxicity in the slice). For experiments where slices were incubated in AF-16 the storage solution also contained 0.5 µg/ml (284 nM) AF-16 (50 µl of a stock solution of AF-16 dissolved in water was added to 100 ml of ACSF). The researcher was blinded on averaged fEPSPs (in the same manner as for input–output measurements). Paired-pulse ratio measurements fEPSPs were evoked at five different stimulation strengths (12 stimuli per stimulation strength) and the magnitudes of the fEPSPs and presynaptic volleys were measured for the averaged potentials for 4 trials per stimulation strength. For each experiment the magnitudes of the fEPSPs were plotted against the magnitudes of the corresponding presynaptic volleys and a linear regression was calculated. The slope of the linear regression line was taken as the input–output measurement for that experiment. Paired-pulse ratio (PPR) measurements were done with an interstimulus interval of 50 ms and these measurements were made on averaged fEPSPs (in the same manner as for input–output measurements).

Stimulation intensity was set by manual inspection of the fEPSP and the intensity never over-reached the point at which population spike activity appeared. In all experiments a test frequency of 0.1 Hz was used. Before tetanizations a stable baseline of 15 min was established (experiments were excluded if fEPSP size changed >10%). LTP was then induced, first by a weak tetanization consisting of a train of 20 impulses at 100 Hz, one stimulation electrode at a time. This was followed by 30 min of test stimulation. After this LTP was induced again by a second strong tetanization consisting of five trains of 20 impulses at 100 Hz given every 20 s, simultaneously in both stimulating electrodes to enhance cooperativity, again followed by 30 min of test stimulation. The amount of potentiation was measured as the change in fEPSP size (average of 20 fEPSPs) at the end of each 30 minutes test stimulation compared to immediately before the first tetanization.

All data are presented as means ± standard error of the mean (SEM) unless stated otherwise. Statistical comparisons were made using nonparametric tests, Mann-Whitney U test or Wilcoxon signed ranks test, and results were considered statistically significant when p < 0.05.

Spatial learning and memory

Spatial learning and memory were tested in the MWM, a 1.6 m in diameter circular pool and a circular platform with a diameter of 10 cm were used. The water was held at 25 ± 1 °C and made opaque by water soluble white paint (Hobbylack matt 250 ml white (#202101), Panduro, Gothenburg, Sweden). The platform was submerged 1 cm below the water surface. The pool was placed in a room with external cues that were kept in constant positions during the whole experiment. Data recordings were made by using the Water Maze Viewer Plug-in (BIOBSERVE, GE).

Rats (SPC-group n = 13 and control-group n = 12) were given an acclimatization training session in the water maze for 30 seconds (s) during which the platform was removed the day before acquisition begun. During the following four days rats had one swim session consisting of twelve swims every day, first and last trials being pre- respective post- probe trials (the platform removed). On the fifth day a single 60 s probe trial was performed. The platform position was held constant within each acquisition day but was changed between acquisition days. Starting position was randomized and varied so that rats were placed in the pool from one of four positions (SW, SE, NE, NW). The rat was always placed in the pool with its nose facing the pool wall and was then allowed to seek the platform for 60 s. If the rat managed to locate the platform within the time limit, it was allowed to rest on the platform for 20 s, if not, the rat was placed upon the platform and got to rest there for 20 s as well. The rat was then put in a plastic cage for 20 s during in which time the pool was cleaned from stools and odors and the swim procedure was then repeated. After each swim session the rat was put back in its home cage. The MWM
paradigm was originally developed by Morris (1984) and adapted by Baldi et al. (2005) to assess short- and long-term memory on each acquisition day. Since the aim of the present study was to assess a potential improvement of cognitive performance in healthy rats, the cognitive demand was increased by varying the platform position between days using the matching-to-place task (Steele and Morris, 1999; Wass et al., 2008). Spatial learning was assessed by recording mean time to find the platform (escape latency) on each of the acquisition days whereas spatial short-term memory was defined as time spent in the target quadrant during the post-probe test and finally, long lasting reference memory was defined as the time spent in the target quadrant on the pre-probe test. To assess development of search strategy, comparisons of mean time to find platform could be assessed across all four test days. Thus, taken together using this MWM paradigm, spatial learning, development of search strategy and spatial short- and long-term (i.e. reference) memory could all be investigated.

All data are presented as means ± SEM. Significance was defined as \( p \leq 0.05 \) using two-tailed levels. Repeated measures two-way ANOVAs with treatment as between-subjects factor and acquisition day/swim number/probe-test day as within-subjects factor was used to analyze mean escape latency/time in target quadrant over the four acquisition days. One-way ANOVAs were used to compare escape latency and time in target quadrant between treatment groups on the different acquisition days. Post hoc pair wise comparisons were Bonferroni corrected.

T-tests were used to compare mean time to find platform on acquisition Day 1 on swim one, and on mean swim speed and average thigmotaxis between treatment groups.

RESULTS

AF-16 enhances LTP in hippocampal CA3-CA1 synapses

AF-16 reduces GABAAergic inhibition onto CA1 pyramidal cells both when applied to hippocampal slices in vitro as well as when endogenous production of AF is upregulated (Kim et al., 2005). This disinhibition could potentially modulate hippocampal synaptic plasticity and in this study we hypothesize that AF enhances LTP and by doing so also enhances learning. To investigate the effect of AF on LTP we performed field recordings from CA3-CA1 glutamate synapses in rat hippocampal slices that had been incubated in AF-16 for \( \geq 1 \) h prior to start of the recording and control slices without added AF-16. A disinhibition of the CA3-CA1 synapses could give rise to a lowering of the induction threshold for LTP as well as an increase in the amount of LTP. Therefor we used two induction protocols in series to induce LTP, a weak tetanization and a strong tetanization (see Experimental procedures). The amount of LTP measured 30 min after the given tetanizations was enhanced in slices incubated in AF-16 both after the weak tetanization (AF-16: 113 ± 2% of baseline, \( n = 8 \), compared to control: 103 ± 2% of baseline, \( n = 7 \), \( p = 0.0037 \), Mann-Whitney U test) as well as after the strong tetanization (AF-16: 133 ± 4% of baseline, \( n = 8 \), compared to control: 111 ± 3% of baseline, \( n = 7 \), \( p = 0.0037 \), Mann-Whitney U test) (Fig. 1). After the weak tetanization there was no significant potentiation compared to baseline in control slices (\( p = 0.089 \), Wilcoxon signed ranks test), whereas the potentiation in AF-16-incubated slices was significant (\( p = 0.012 \)). There was no significant difference in fiber volley magnitude between the two groups (after weak tetanization, AF-16: 98 ± 2% of baseline, \( n = 8 \), compared to control: 100 ± 2% of baseline, \( n = 7 \), \( p = 0.19 \), Mann-Whitney U test, and after strong tetanization, AF-16: 98 ± 1% of baseline, \( n = 8 \), compared to control 97 ± 1% of baseline, \( n = 7 \), \( p = 0.78 \), Mann-Whitney U test).

Endogenously upregulated AF by SPC-feeding enhances LTP in CA3-CA1 synapses

Next, we investigated if endogenously upregulated AF in rats fed with SPC also enhances LTP. To assess if SPC treatment alters any basic synaptic properties we first measured input–output relationship and PPR of the CA3-CA1 synapses in hippocampal slices from rats fed with SPC compared to control rats. There were no significant differences in neither input–output relationship (measured as slope of linear regression, see Experimental procedures; SPC: 0.55 ± 0.1, \( n = 7 \), compared to control: 0.47 ± 0.1, \( n = 9 \), \( p = 0.41 \), Mann Whitney U test) (Fig. 2A) nor PPR (SPC: 1.60 ± 0.04, \( n = 7 \), compared to control: 1.55 ± 0.05, \( n = 9 \), \( p = 0.35 \), Mann-Whitney U test) (Fig. 2B). In slices from rats with endogenously upregulated AF levels the amount of LTP was enhanced both after a weak tetanization (SPC: 115 ± 2% of baseline, \( n = 10 \), compared to control: 107 ± 2% of baseline, \( n = 10 \), \( p = 0.035 \), Mann-Whitney U test) as well as after a strong tetanization (SPC: 136 ± 7% of baseline, \( n = 10 \), compared to control 117 ± 3% of baseline, \( n = 10 \), \( p = 0.023 \), Mann-Whitney U test) (Fig. 3). After the weak tetanization there was a significant potentiation compared to baseline in both control slices and in slices from rats fed with SPC (\( p = 0.022 \) and \( p = 0.0051 \), respectively, Wilcoxon signed ranks test). There was no significant difference in fiber volley magnitude between the two groups (after weak tetanization, SPC: 99 ± 1% of baseline, \( n = 10 \), compared to control: 99 ± 2% of baseline, \( n = 10 \), \( p = 0.85 \), Mann-Whitney U test).

AF-16 does not enhance LTP when GABAA receptors are blocked by PTX

To test the effect of AF on LTP when blocking GABAAergic transmission, we also performed LTP measurements with the same protocol as previously but in the presence of the GABAA receptor channel blocker PTX. In the presence of PTX there was no significant difference in the amount of LTP neither after a weak tetanization (AF-16: 118 ± 5%
Spatial learning improved over test days and was improved by SPC treatment

The potential effects of SPC treatment on spatial learning and memory were investigated in the water maze task (Morris, 1984) as adapted by Baldi et al. (2005) with changes in platform position between days to increase cognitive demand (Steele and Morris, 1999; Wass et al., 2008).

Importantly, on day one, there was no difference in time to find platform between treatment groups on acquisition swim number one. Thus, there were no baseline differences between treatment groups in finding the platform before any acquisition training had occurred. In addition, no significant difference between the groups was detected regarding swim speed on the different acquisition days (Fig. 5A) and average thigmotaxis over the four acquisition days.

There was a significant main effect of acquisition day: $F(3, 69) = 25.89, p < 0.001$ on escape latency over the four acquisition days such that learning occurred in both treatment groups (Fig. 5B). Post hoc Bonferroni corrected analysis found significant differences in mean acquisition latency on Day 1 compared to; Day 2 ($p < 0.05$); Day 3 ($p < 0.001$); Day 4 ($p < 0.001$) as well as between Day 2; and Day 3 ($p < 0.05$) and Day 4 ($p < 0.001$), such that escape latency decreased over days, indexing spatial learning. There was a main effect of treatment: $F(1, 23) = 4.19, p = 0.05$ such that SPC treated animals displayed a shorter escape latency compared to controls. Repeated measures one-way ANOVA, between subject comparisons, found significant shorter escape latency for SPC treated animals on Day 1 ($p = 0.04$) and Day 4 ($p = 0.03$) (Fig. 5B). No significant interaction effect between treatment and day was identified.

To further investigate the effect of SPC-treatment on spatial learning, time to find platform over the 10 swims was compared between the groups on each day. On Day 1, there was a significant main effect of treatment $F(1, 23) = 4.59, p = 0.04$ such that SPC treated animals displayed a shorter mean time to find the platform compared with controls, between subject comparisons found significantly shorter latencies in SPC treated animals on swim number 4 ($p = 0.01$) and swim number 8 ($p = 0.003$). A significant main effect of swim number $F(5.95, 136.90) = 9.33, p < 0.001$ as well as between swim number 2 and; 5, 9 and 10 ($p < 0.05$); 7 ($p < 0.01$); and Day 4 ($p < 0.001$) such that escape latency decreased over days, indexing spatial learning. There was a main effect of treatment: $F(1, 23) = 4.19, p = 0.05$ such that SPC treated animals displayed a shorter escape latency compared to controls. Repeated measures one-way ANOVA, between subject comparisons, found significant shorter escape latency for SPC treated animals on Day 1 ($p = 0.04$) and Day 4 ($p = 0.03$) (Fig. 5B). No significant interaction effect between treatment and day was identified.
F(1, 23) = 5.13, p = 0.03, such that SPC treated animals had shorter swim latencies. A trend towards an interaction effect between swim number and treatment, F(3.66, 84.14) = 2.32, p = 0.07, was found (Fig. 7). Between subject comparisons found that SPC treated animals had significantly shorter latencies on swim 1 (p = 0.03) compared with placebo treated animals. Significant improvement in mean time to find platform during Day 4 was detected between swim number 1 and; 4 and 8 (p < 0.05); 5 and 9 (p < 0.01) as well as swim number 2 and 8 and 9 (p < 0.05).

Post-probe performance improved over test days and was enhanced by SPC treatment on day 1

A trend towards a main effect of the treatment; F(1, 21) = 3.51, p = 0.08 and a significant effect of day; F (3, 63) = 13.77, p < 0.001 on time spend in the target quadrant during the post-probe test and a significant interaction between day and treatment F(3, 63) = 3.34, p = 0.03, was identified (Fig. 8A). SPC treated animals spent more time in the target quadrant, compared with placebo animals on Day 1 (p = 0.03) and a trending effect on Day 3 (p = 0.06). Post hoc pairwise comparisons identified significant differences between Days 1 and 4 (p = 0.01), 2 and 4 (p = 0.01) and between 3 and 4 (p < 0.001), such that animals spent more time in the target quadrant on Day 4 compared to all other days (Fig. 8A).

Pre-probe performance improved over test days but was not affected by SPC treatment

On the pre-probe test, a main effect of day F(3, 69) = 17.02, p < 0.001, but no effect of treatment nor any interaction between treatment and day, were found. Effect of day was such that animals spent more time in the previous target quadrant on day 5 (p = 0.002) compared with day 2, on day 3 compared to day 4 (p < 0.001) and on day 5 compared to day 4 (p < 0.001), indicating formation of long-term memory in both treatment groups (Fig. 8B).

DISCUSSION

We have previously shown that the endogenous peptide AF-16 apart from its antisecretory and anti-inflammatory effects also modulates GABAergic transmission in the rat hippocampus in such a way that it produces disinhibition of CA1 pyramidal neurons (Kim et al., 2005; Strandberg et al., 2014). Such disinhibition could potentially alter the threshold for induction of, and/or the amount of LTP (Wigstrom and Gustafsson, 1983) and therefore potentially also enhance hippocampus dependent learning (Nicoll, 2017; Mansvelder et al., 2019). In
this study we investigated the effects of AF-16 on LTP in CA3-CA1 synapses and the effect of enhancing endogenous production of AF on spatial learning. We found that AF enhances LTP at hippocampal CA3-CA1 synapses, promotes spatial learning and enhances spatial short-term memory, but had no effect on spatial long-term memory.

AF applied in vitro, as well as when upregulated endogenously by SPC-feeding, enhanced both the small and largely decaying LTP after a weak tetanization as well as LTP after a strong tetanization. These results demonstrate that AF facilitates the induction of LTP and enhances the magnitude of LTP. Such an enhancement of LTP is fully consistent with a disinhibitory effect of AF (Wigstrom and Gustafsson, 1983; Hanse and Gustafsson, 1992). We obtained direct support for this conclusion by showing that AF did not facilitate LTP in the presence of the GABA_A receptor blocker PTX. Moreover, we have previously shown that AF causes disinhibition of CA1 pyramidal cells (Kim et al., 2005). Intriguingly, this disinhibition is likely mediated by an AF-induced up-regulation of tonic GABAergic signaling in inhibitory interneurons (Strandberg et al., 2014). Thus, we propose a scenario for facilitating LTP by an up-regulation of tonic GABAergic signaling on inhibitory interneurons. Such a scenario has previously been described in the amygdala where knocking out the GABA_A δ subunit, which mediates tonic inhibition of interneurons in the lateral amygdala, decreased disinhibition onto projection neurons and thereby inhibited LTP in these neurons, and by doing so impaired fear learning (Liu et al., 2017). Thus, our results from the hippocampus add to the concept that tonic inhibition of inhibitory interneurons provides a powerful modulatory mechanism regulating learning. The cellular mechanisms by which AF promotes the increased tonic GABAergic signaling specifically on inhibitory interneurons are not known. It is, however, interesting that this facilitation of LTP can be induced solely by a specific combination of carbohydrates and amino acids. Indeed, we found similar facilitation of LTP in SPC-fed animals as with acute treatment of hippocampal slices with the AF peptide. Based on previous studies (Rapallino et al., 2003; Kim et al., 2005; Strandberg et al., 2014; Bazzurro et al., 2018), we used a single concentration
We have measured the amount of LTP 30 minutes after its induction, which might seem a rather early time given that LTP could last for, at least, hours in vitro. Thus, our conclusion that AF facilitates the induction of LTP (via disinhibition) cannot be extended to what sometimes is referred to as late LTP. Different induction protocols are typically used to induce LTP of different durations. For example, repeated strong tetanizations are often used to induced long-lasting LTP, whereas brief weaker tetanization is used to induce more transient LTP. In this study, we used one such weak induction and one stronger induction of LTP, both of which were facilitated by AF.

When endogenously upregulating AF and thereby enhancing LTP in the hippocampus one might expect alterations in long-term synaptic strength. An increased synaptic strength would give a greater synaptic response upon a given presynaptic activation and this should increase the input–output relationship. We did not, however, observe any significant difference in input–output relationship between SPC-fed rats and controls. This could be because of the sample size being too small and that we were not able to detect a small difference, but maybe more likely due to a homeostatic synaptic scaling, a process in where the...
During the post-probe test, a significant effect of day **p < 0.001, but no effect of treatment, but a significant interaction between day and treatment p = 0.03, was found. Pair wise comparisons found effect of treatment on Day 1 *p < 0.03. During the pre-probe test, a significant effect of day ***p < 0.001, but no effect of treatment was found.

In the present study, platform position was altered between days in order to make the task more cognitively demanding. Healthy rats quickly learn to navigate spatially in the MWM and thus it is unlikely that improving cognitive functioning by increasing AF-16 would have been detected. As such, in the paper by Clausen et al. (2017) controls rats, as well as AF-16-only treated rats, improved their spatial learning equally and substantially between day 1 (mean latency to platform around 50 seconds S)) and day 2 (mean latency around 15 s), and after that, time to find platform was more or less stationary around 10 s day 3 and day 4, thus there was not much room for improvement of AF-16 on spatial learning. Also, the probe test (assessing long term memory) was carried out 72 h after the last training session in Clausen et al.’s (2017) study. In our study, probe testing, indexing long term memory, was carried out in the morning, i.e. within 24 h, after training session. However, neither study found an effect of AF-16 on long-term spatial memory in healthy animals. Given that AF-16 in fact seems to enhance LTP in the CA1 hippocampal region, as found herein, it was hypothesized that SPC treatment would have enhanced long term spatial memory in the MWM. However, and again, healthy rats seem to acquire a strategy for remembering platform position from the previous day, as we did find a significant improvement in time spent in target quadrant on the pre-probe test Day 4 compared to all other test days, see Fig. 8B. Thus, it is possible that a ceiling effect was reached in healthy control rats, why no such improvement would have been detected had there been one. It is also possible that our protocol is not optimal for assessing long-term spatial memory, or there is no such effect of AF-16 on spatial memory.

The disinhibitory effect of AF described here, mediated by decreasing interneuron excitability through

---

**Fig. 8.** Time spent in target quadrant during post- and pre-probe improved over days and was enhanced by SPC on post-probe Day 1. Box plots depicting time (in seconds) spent in target quadrant during post- and pre-probe tests, over test days. (A) During the post-probe test, a significant effect of day **p < 0.001, but no effect of treatment, but a significant interaction between day and treatment p = 0.03, was found. Pair wise comparisons found effect of treatment on Day 1 *p < 0.03. (B) During the pre-probe test, a significant effect of day ***p < 0.001, but no effect of treatment was found.
an interneuron-specific upregulation of tonic GABAergic inhibition (Kim et al., 2005; Strandberg et al., 2014), adds another mechanistic scenario to previously described mechanisms of disinhibition in the hippocampus which include various examples of inhibition of interneurons by other interneurons in the cortical network (Letzkus et al., 2015; Artinian and Lacaille, 2018). This AF-mediated disinhibition, which enhanced hippocampal LTP and learning as well, can readily be achieved by adding SPC to the food. This is quite intriguing since SPC-Flakes® actually are available for humans as a “Food for specific medical purposes” and AF-16 has been clinically tested according to the regulatives of a phase I study and might develop into a certified medical drug in the future. Whether enhancing AF activity in humans have any effect on LTP and learning is however unclear, but it could potentially be beneficial in neuro-rehabilitation and/or neurodegenerative diseases.

ACKNOWLEDGMENTS

This work was supported by research grants issued by the Alzheimerfonden (AF-640391 to E.H.), Hjärnfonden (FO2021-0048 to E.H.), Magnus Bergvall’s Stiftelse (2014-00256 to J.S.), Swedish Research Council 2020 (00878 to E.H.), Swedish State Support for Clinical Research (ALFGBG 427611 to E.H.) and Ahlén-stiftelsen (mC32/h14 to J.S.).

AUTHORS CONTRIBUTIONS

C.W., E.H. and J.S. conceived and designed experiments. L.W. and S.K. performed experiments. C.W., J.S., L.W. and S.K. analyzed the data. C.W., E.H., J.S., L.W. and S.L. wrote the paper. All authors read, commented on and approved the final draft of the paper.

DECLARATIONS OF INTEREST

None.

REFERENCES

Al-Olama M, Lange S, Lönroth I, Gatzinsky K, Jennische E (2015) Uptake of the antisecretory factor peptide AF-16 in rat blood and cerebrospinal fluid and effects on elevated intracranial pressure. Acta Neurochir (Wien) 157(1):129–137.

Al-Olama M, Wallgren A, Andersson B, Gatzinsky K, Hultborn R, Karlsson-Parra A, Lange S, Hansson H-A, Jennische E (2011) The peptide AF-16 decreases high interstitial fluid pressure in solid tumors. Acta Oncol 50(7):1098–1104.

Artinian J, Lacaille JC (2018) Disinhibition in learning and memory circuits: New vistas for somatostatin interneurons and long-term synaptic plasticity. Brain Res Bull 141:20–26.

Baldi E, Efoudebe M, Lorenzini CA, Bucherei C (2005) Spatial navigation in the Morris water maze: working and long lasting reference memories. Neurosci Lett 378(3):176–180.

Bazzurro V, Gatta E, Cupello A, Lange S, Robello M (2018) Antisecretory Factor Modulates GABAA Receptor Activity in Neurons. J Mol Neurosci 64(2):312–320.

Björck S, Bosaeus I, Ek E, Jennische E, Lönroth I, Johansson E, Lange S (2000) Food induced stimulation of the antisecretory factor can improve symptoms in human inflammatory bowel disease: a study of a concept. Gut 46:824–829.

Clausen F, Hansson HA, Raud J, Marklund N (2017) Intranasal Administration of the Antisecretory Peptide AF-16 Reduces Edema and Improves Cognitive Function Following Diffuse Traumatic Brain Injury in the Rat. Front Neurol 8:39.

Davidson TS, Hickey WF (2004) Distribution and immunoregulatory properties of antisecretory factor. Lab Invest 84(3):307–319.

Eriksson A, Shafazand M, Jennische E, Lange S (2003) Effect of antisecretory factor in ulcerative colitis on histological and laboratory outcome: a short period clinical trial. Scand J Gastroenterol 38(10):1045–1049.

Finkel Y, Bjarnason I, Lindblad A, Lange S (2004) Specially processed cereals: a clinical innovation for children suffering from inflammatory bowel disease? Scand J Gastroenterol 39(1):87–88.

Gatzinsky K, Johansson E, Jennische E, Oshalmi M, Lange S (2020) Elevated intracranial pressure after head trauma can be suppressed by antisecretory factor-a pilot study. Acta Neurochir (Wien) 162(7):1629–1637.

Goransson L, Martinsson K, Lange S, Lönroth I (1993) Feed-induced lectins in piglets. Feed-induced lectins and their effect on post-weaning diarrhoea, daily weight gain and mortality. Zentralbl Veterinarmed B 40:478–484.

Hanner P, Rask-Andersen H, Lange S, Jennische E (2010) Antisecretory factor-inducing therapy improves the clinical outcome in patients with Meniere’s disease. Acta Otolaryngol 130:223–227.

Hansson E, Gustafsson B (1992) Postsynaptic, but not presynaptic, activity controls the early time course of long-term potentiation in the dentate gyrus. J Neurosci 12(8):3226–3240.

Ilkhanizadeh S, Sabelström H, Miroshnikova YA, Frantz A, Zhu W, Idilli A, Lakins JN, Schmidt C, Quigley DA, Fenster T, Yuan E, Trzeiczak JR, Saxena S, Lindberg OR, Mouw JK, Burdick JA, Magnitsky S, Berger MS, Phillips JJ, Arosio D, Sun D, Weaver VM, Weiss WA, Persson AI (2018) Antisecretory Factor-Mediated Inhibition of Cell Volume Dynamics Produces Antitumor Activity in Glioblastoma. Mol Cancer Res 16(5):777–790.

Jennische E, Bergström T, Johansson M, Nyström K, Tarkowski A, Hansson HA, Lange S (2008) The peptide AF-16 abolishes sickness and death at experimental encephalitis by reducing increase of intracranial pressure. Brain Res 1227:189–197.

Johansson E, Al-Olama M, Hansson HA, Lange S, Jennische E (2013) Diet-induced antisecretory factor prevents intracranial hypertension in a dosage-dependent manner. Br J Nutr 109 (12):2247–2252.

Johansson E, Lange S, Bergström T, Oshalmi M, Lönroth I, Studahl M (2018) Increased level of complement in cerebrospinal fluid of patients with herpes simplex encephalitis. J Neurovirol 24 (6):702–711.

Johansson E, Lange S, Jennische E (2011) Specially processed cereals diet increases plasma levels of active antisecretory factor and up-regulates rat hepatic glutathione S-transferase mu. Nutrition 27(9):949–954.

Johansson E, Lange S, Lönroth I (1997) Identification of an active site in the antisecretory factor protein. Biochim Biophys Acta 1362 (2-3):177–182.

Johansson E, Lönroth I, Jonson I, Lange S, Jennische E (2009) Development of monoclonal antibodies for detection of Antisecretory Factor activity in human plasma. J Immunol Methods 342(1-2):64–70.

Johansson E, Lönroth I, Lange S, Jonson I, Jennische E, Lönroth C (1995) Molecular cloning and expression of a pituitary gland protein modulating intestinal fluid secretion. J Biol Chem 270 (35):20615–20620.

Kaya I, Johansson E, Lange S, Malmberg P (2017) Antisecretory Factor (AF) egg-yolk peptides reflects the intake of AF-activating feed in hens. Clin Nutr Exp 12:27–36.

Kim M, Wasling P, Xiao M-Y, Jennische E, Lange S, Hansa E (2005) Antisecretory factor modulates GABAergic transmission in the rat hippocampus. Regul Pept 129(1-3):109–118.

Lange S, Hultborn R, Jennische E (2020) Antisecretory factor AF-16 improves vascular access to a rat mammary tumour. APMIS 128 (5):387–389.
Lange S, Jennische E, Johansson E, Lonnroth I (1999) The antisecretory factor: synthesis and intracellular localisation in porcine tissues. Cell Tissue Res 296:607–617.

Lange S, Lonnroth I (2001) The antisecretory factor: synthesis, anatomical and cellular distribution, and biological action in experimental and clinical studies. International review of cytolgy 210:39–75.

Laurenius A, Wängberg B, Lange S, Jennische E, Lundgren BK, Bosaeus I (2003) Antisecretory factor counteracts secretory diarrhoea of endocrine origin. Clin Nutr 22(6):549–552.

Letzkus J, Wolff SE, Lüthi A (2015) Disinhibition, a Circuit Mechanism for Associative Learning and Memory. Neuron 88(2):264–276.

Liu Z-P, He Q-H, Pan H-Q, Xu X-B, Chen W-B, He Ye, Zhou J, Zhang W-H, Zhang J-Y, Ying X-P, Han R-W, Li B-M, Gao T-M, Pan B-X (2017) Delta Subunit-Containing Gamma-Aminobutyric Acid A Receptor Disinhibits Lateral Amygdala and Facilitates Fear Expression in Mice. Biol Psychiatry 81(12):990–1002.

Lonnroth I, Oshalim M, Lange S, Johansson E (2016) Interaction of Proteasomes and Complement C3, Assay of Antisecretory Factor in Blood. J Immunoassay Immunochem 37(1):43–54.

Mansvelder HD, Verhoog MB, Goriounova NA (2019) Synaptic plasticity in human cortical circuits: cellular mechanisms of learning and memory in the human brain? Curr Opin Neurobiol 54:186–193.

Matson Dzebo M, Reymer A, Fant K, Lincoln P, Nordén B, Rocha S (2014) Enhanced cellular uptake of antisecretory peptide AF-16 through proteoglycan binding. Biochemistry 53(11):6566–6573.

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11(1):47–60.

Nawrot-Porąbka K, Jaworek J, Leja-Szpak A, Kot M, Lange S (2015) The role of antisecretory factor in pancreatic exocrine secretion: studies in vivo and in vitro. Exp Physiol 100(3):267–277.

Nicolas V, Liévin-Le Moal V (2015) Antisecretory factor peptide AF-16 inhibits the secreted autotransporter toxin-stimulated transcellular and paracellular passages of fluid in cultured human enteroocyte-like cells. Infect Immun 83(3):907–922.

Nicoll RA (2017) A Brief History of Long-Term Potentiation. Neuron 93(2):281–290.

Rapallino MV, Cupello A, Lange S, Lonnroth I (2003) Antisecretory factor peptide derivatives specifically inhibit [3H]-gamma-aminobutyric acid(36Cl-) out–> in permeation across the isolated rabbit Deiters’ neuronal membrane. Acta Physiol Scand 179:367–371.

Steele RJ, Morris RGM (1999) Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. Hippocampus 9(2):118–136.

Strandberg J, Lindquist C, Lange S, Asztely F, Hanse E (2014) The endogenous peptide antisecretory factor promotes tonic GABAergic signaling in CA1 stratum radiatum interneurons. Front Cell Neurosci 8:13.

Svensson K, Lange S, Lonnroth I, Widstrom AM, Hanson LA (2004) Induction of anti-secretory factor in human milk may prevent mastitis. Acta Paediatr 93:1228–1231.

Tononi G, Cirelli C (2020) Sleep and synaptic down-selection. Eur J Neurosci 51(1):413–421.

Wass C, Svensson L, Fejgin K, Palsson E, Archer T, Engel JA, Kliamer D (2008) Nitric oxide synthase inhibition attenuates phencyclidine-induced disruption of cognitive flexibility. Pharmacol Biochem Behav 89(3):352–359.

Wigstrom H, Gustafsson B (1983) Facilitated induction of hippocampal long-lasting potentiation during blockade of inhibition. Nature 301(5901):603–604.

Zaman S, Aamir K, Hanson LA, Lange S (2018) High doses of Antisecretory Factor stop diarrhea fast without recurrence for six weeks post treatment. Int J Infect Dis 71:48–52.