Glucose-monitoring neurons of the nucleus accumbens in the central regulation of feeding

Ph.D. thesis

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I. Introduction

The incidence of feeding and metabolism associated diseases is rapidly increasing all around the world. Due to their importance with respect to public health, the better understanding of processes in the background of cachexia and obesity, anorexia and bulimia nervosa, various metabolic disturbances such as diabetes mellitus and metabolic syndrome is indispensable in order to develop effective therapeutic strategies. Data show altered central regulation of the homeostatic functions in these diseases, and it has also been suggested that some symptoms occur as a result of regulatory abnormalities. Considering the above, it is substantially important to unravel more and more details of the central homeostatic regulation.

In this regard, neurons of the nucleus accumbes, a key structure in the forebrain limbic circuitry, could play especially significant role. The present experiments were designed to achieve a better understanding of particular roles of these neurons in the central control of feeding and metabolism.

Several interconnected peripheral and central neural and humoral-metabolic regulatory processes take part in the maintenance of homeostasis, the stable internal environment of organism. Such processes regulate the adequate food and fluid intake, the plasma glucose and metabolite concentrations, the metabolism and energy balance and the appropriate level of the defense functions of the organism, in general, mechanisms associated to these processes are pivotal in the motivational, perceptual and cognitive functions as well.

Numerous brain nuclei and neurotransmitter fiber systems have been shown to be of distinguished significance in the regulation of food intake and body weight during the last 100 years. In addition to the so-called ”hunger center” in the lateral hypothalamic area (LHA) [1-4] and the so-called ”satiety-center” in the ventromedial hypothalamic nucleus (VMH) [1,4,5], several other extrahypothalamic structures, such as the amygdala (AMY) [6,7], the mediodorsal prefrontal (mdPFC) and the orbitofrontal (OBF) cortices [8-16], furthermore the globus pallidus (GP) [17-20] and brain stem nuclei also play important role in the central regulation of feeding [21].

In the recent decades, several characteristic neurotransmitter pathways whose damage greatly influence food intake behavior have also been identified. Amond these, the most important ones in terms of feeding control are the catecholaminergic tracts, the ventral

noradrenergic bundle (VNAB) originating in the locus ceruleus (LC), and called as ”satiety pathway” [22], the nigrostriatal dopaminergic system (NSDS) originating in the substantia nigra, and the mesolimbic dopaminergic system (MLDS) projecting from the ventral tegmental area, and called as ”hunger pathway” [23-26].

Both the peripheral and central glucoreception play important role in the regulation of food intake [27]. The shifts of the brain's interstitial glucose concentration following the change of blood glucose levels are detected by a particular of neurons group, the so called glucose-monitoring (GM) neural cells [1,28-31]. In addition to the hypothalamus, the presence of these neurons is verified in the NTS, the area postrema, the AMY, moreover, in the globus pallidus and in the prefrontal-orbitofrontal cortex as well [1,28,30-47]. These multifunctional chemosensory neurons are able to recognize the current humoral changes of the internal environment [1,32,47-51], furthermore, they take part in the feeding associated sensory-motor, perceptual-motivational integration and in related learning and memory functions as well [30,33,38,39,52-60]. The GM neurons can be divided into two groups on the basis of their morphological and functional characteristics: the large, multipolar glucose-receptor (GR) units that increase, and the small, longitudinal multi-, or bipolar glucose-sensitive (GS) cells that decrease their firing rate in response to elevation of blood glucose level. The glucose insensitive (GIS) neurons use the glucose only in their metabolism, and not in the mediation of neural information. [1].

Cells of peripheral organs and central nervous system neurons involved in the control of feeding behavior show striking analogy with respect to their sensing mechanisms of the extracellular glucose concentration. It has been demonstrated that a hexokinase basically identical to that in the pancreatic β-cells could also be found in certain CNS neurons whose localization is the same as that of a group of the GM cells [61,62]. Furthermore, the presence of ATP-sensitive potassium channels (K_{ATP}) so important in the activation of β-cells has also been demonstrated in the GM neurons [63-65]. In addition, the type 2 glucose transporter protein (GLUT2) that takes glucose into the pancreatic β-cells was demonstrated to be functional in the CNS, and its neural expression was proven in GM cell containing brain regions, such as the hypothalamus and the OBF [66-68]. On the basis of the above similarities it is suggested that a special toxin called streptozotocin (STZ) - which can destroy the pancreatic β-cells, has an analogous molecular structure to the glucose, and is produced by the fungus *Streptomyces achromogenes* - could be utilized in our experiments for selective destruction of the GM neurons.

Based on the close relationship of the nucleus accumbens (NAcc), a key constituent of the forebrain limbic circuitry and known to play an important role in the central feeding control [69-79], with the GM neuron containing brain structures it is reasonable to suppose the complex chemosensitivity of accumbens neurons. The NAcc is located in the basal forebrain, among others it functions as the integrating nucleus of the striatopallidal system. From neuroanatomical point of view it can be divided into three main parts: the so called shell, core and rostral divisions [77,80]. Afferent fibers from the shell project to the ventral pallidum, lateral preoptic nucleus, LHA, VTA, central periaqueductal gray, nuclei of the brain stem and to the cervical spinal cord [77-79,81], and *vice versa*, efferent fibers from all these structures return here. The fibers that originate in the core subregion of the NAcc project mainly to the dorsolateral part of the ventral pallidum, to the entopeduncular nucleus, SN and to the medial part of the subthalamic nucleus [77]. The fibers originating in the third, controversial rostral subdivision of the NAcc project essentially to the same destination as the fibers from the shell and core [77,81]. In the background of the multiple roles exerted by the NAcc in the regulation of hunger motivated behavior several neurotrasmitter mechanisms are postulated. [71,72]. The NAcc has glutamatergic, noradrenergic, dopaminergic, serotoninergic, opiatergic and also histaminergic innervation. Its most important excitatory (glutamatergic) afferents arrive from the mediodorsal thalamic nucleus (MD), the prefrontal cortex, basolateral amygdala and from the hippocampus [82,83]. The accumbens receives abundant dopaminergic input from the VTA [84,85], noradrenergic fibers from the locus ceruleus [86], serotoninergic fibers from the raphe nuclei [87], and histaminergic ones from the tuberomamillary nucleus [88]. Data in the literature clearly show that the two subdivisions of the NAcc play differential roles in the central feeding control. The inhibition of or damage to the shell region reportedly cause a robust food intake and increase of body weight [71,89], whereas excitotoxic lesion of the core region results in a substantial decrease of feeding motivation with a parallel decrease of body weight [71,90]. It is also known that both subdivisions take part in taste-associated motivational and learning mechanisms [72].

Our experiments were designed to elucidate in more details the complex roles of accumbens neurons in the central regulation of feeding.

II. Experiments

1. Questions

Our research project related to this doctoral thesis had two main directions. On the one hand, extracellular single neuron activity was recorded by means of tungsten wired multibarreled glass microelectrodes, and the glucose sensitivity of NAcc neurons was explored in adult, anesthetized Wistar rats and alert rhesus monkey. The endogenous (to neurotransmitters and neuromodulators) and the exogenous (to tastes) chemosensitivity of these neurons was examined in our experiments.

On the other hand, the effect of GM cells of the rodent NAcc on behavioral and metabolic processes was investigated in the other major line of our studies. We have tried to verify the homeostatic significance of these chemosensory neurons by elucidating the consequences of their selective destruction. For this purpose, based on data of literature and the findings of our preliminary experiments, STZ was administered into the NAcc of laboratory rats, and effects of this microinjection on the blood glucose level in relation to a glucose tolerance test /GTT/), plasma cholesterol, HDL, LDL, triglyceride and uric acid concentrations were measured. The food and fluid intake associated behavioral effects of the STZ microinjection into the NAcc were studied in conditioned taste aversion and taste-reactivity paradigms.

The present experients were performed to answers the following questions:

I. In rodent and primate microelectrophysiological experiments, using the multibarreled microelectrophoretic technique, it was examined that:

1. Do nucleus accumbens neurons change their activity to microelectroosmotic administration of D-glucose, that is, it was to decide whether GM neurons could be identified in this forebrain limbic structure?

2. Is the activity of these GM cells influenced by various neurotransmitters and neuromodulators (DA, GABA, Ach, DA-antagonists), whose majority is present in the NAcc even in natural circumstances, and furthermore, by STZ, which proved to be toxic for the GM neurons in our previous experiments?

3. Do taste stimuli, essential in guiding food and fluid intake, change the firing rate of GM cells in the NAcc?

II.a. Behavioral-biochemical experiments in the laboratory rat to explore the metabolic consequences of STZ microinjection into the NAcc, were conducted to elucidate that:

1. Has the selective destruction of GM cells any effect on the carbohydrate metabolism, does it influence the glucose tolerance of the animals?
2. Has the specific lesion of these chemosensory neurons any effect on relevant plasma metabolite levels (cholesterol, HDL, LDL, triglycerides, uric acid)?

II.b. In other behavioral experiments, to unravel the effect of STZ microinjection into the nucleus accumbens on gustatory functions, it was investigated whether selective lesioning of the GM cells:

1. Can modify taste-associated aversive learning ability of the animals?
2. Can result in taste perception alteration in taste reactivity tests?

2. Methods

2.1 Animals

Altogether 136 adult male Wistar rats with an average body weight of 293 ± 36 g at the beginning of the study were used in our experiments. In the microelectrophysiological investigations, an adult male rhesus monkey (*Macaca mulatta*) was also used. Both the rodents and the monkey were kept in separated, individual cages throughout the experiments. Unless where it is stated different, they could eat and drink freely. In the rodent room, 12-12 hours long light-dark cycle was employed with an illumination close or identical to that of the wave-length of the natural light. In the monkey room, during the 12 hours long light period, the natural illumination was complemented with a similar wave-lenght light-source. In the animal rooms, the temperature (23-25 °C), and the humidity (55-65%) were kept in a constant range. Animals were kept and all experimental procedures were conducted in accordance with institutional, national and international regulations.

2.2 Electrophysiological experiments

2.2.1 Surgery

During the experiments with rats ketamine or urethane anesthesia was used. The head of the rats, placed in a stereotaxic instrument, was fixed, their scalp incised, and a small (2–3mm in diameter) hole was drilled through the cleaned skull. The dura was finely incised (0.5–1 mm), and the electrode was led to the accumbens by means of a hydraulic microdrive (Narishige MO-10, Japan) under microscopic control. The stereotaxic coordinates of extracellular single neuron recordings were as follows: anteroposterior, bregma + 3.2–3.7 mm; mediolateral, 1.2–1.6 mm; vertical, 5.6–7.6 mm from surface of the brain, according to the stereotaxic rat brain atlas of Pellegrino et al [91].

In case of the monkey, an aseptic operation under ketamine introduced sodium-pentobarbital anesthesia was performed to bild up the acrylate head piece which allowed us to fix the monkey's head in a stereotaxic apparatus during the electrophysiological experiments. Throughout the single neuron recordings the animal was sitting in a special „primate chair". Positioning of the electrode was performed the same way as described in case of the rats, with the exception of that the monkey was alert during the whole recording experiment. The stereotaxic coordinates were as follows: AP, 19.75-25.35 mm; ML, 1.35-3.8 mm; V: 27.2-30.2 mm [92,93].

2.2.2 Extracellular single neuron recording

For the extracellular single neuron recording, tungsten wired multibarreled glass microelectrodes, manufactured by ourselves, were employed. Extracellular action potentials of the neurons were recorded by the tungsten wire containing central barrel of the electrode. The 8-10 tiny capillaries that surrounded the central barrel were used to deliver the various chemicals to the vicinity of the recorded neuron. The action potentials were passed into a preamplifier, then to a high gain amplifier. Standard pulses were formed by means of low and high cut filters and a window discriminator (Supertech Ltd., Hungary). Analogue signals were also led to a microprocessor controlled A/D converter device (CED 1401plus, Spike 2 software package, UK). The spike discharges and pulses were continuously monitored on oscilloscopes (HAMEG HM-

2035 and HM-2037, Germany). Raw data, formed pulses and marker signals were all fed into a microcomputer and stored on hard disk and compact disks for off-line analyses. Only the activity of spontaneously firing, well-isolated cells has been recorded. Our system enabled us to record and analyze ECG and respiration as well.

2.2.3 Neurochemical experiments – Taste-stimulation

The capillaries surrounding the central barrel of the tungsten wired multibarreled glass microelectrodes were filled with the following solutions: 0.5 M D-glucose, 0.5 M dopamine HCl, 0.01 M SCH-23390, 0.01 M sulpiride, 0.5 M acethylcholine HCl, 0.5 M γ-amino-butiric acid, 0.0037 M STZ, 0.15 M NaCl (balance channel), and 0.5 M monosodium L-glutamate (MSG). Microelectrophoretic applications were performed by delivering constant currents (in the 10–90 nA range) of appropriate polarity to eject the chemicals from their respective barrels (NeuroPhore BH-2 System, USA). During the recording experiments, the taste responsiveness of neurons was also examined by taste stimulations via an intraorally implanted cannula. In accordance with the international standards, the following taste solutions were tested: sweet (sucrose; 0.1M and 0.3M), salty (NaCl; 0.1M and 0.3M), sour (HCl; 0.01M and 0.03M), bitter (QHCl; 0.001M and 0.003M) and umami (MSG; 0.1M és 0.3M). As complex taste orange juice (10% and 25%) was used.

2.3 Metabolic and behavioral experiments

2.3.1 Surgery

The guide cannulas that enabled the targeted intracerebral microinjection were implanted during a stereotaxic operation performed under ketamine anesthesia. The stereotaxic coordinates were: AP, Bregma + 3.6 mm; ML 1.3 mm [91]. The cannulas were placed bilaterally right above the NAcc on the surface of the dura mater, and were cemented to the skull by dental acrylic.

2.3.2 Nucleus accumbens microinjections

Microinjections were directly made into the NAcc. Alert, well-handled rats were kept calm in hand and at both sides a 30G stainless steel delivery cannula, connected with a 12-15 cm long PE tube to a 25 µl Hamilton syringe, was led through the guide cannula to the NAcc (AP, B + 3.6 mm; ML, 1.3 mm; V, 5.75 mm). The syringe was filled with STZ or physiological saline as control. A microinfusion pump (M101, Stoelting, USA) was employed to deliver the solutions.

2.3.3 Metabolic experiments

The effect of accumbens STZ microinjection on the carbohydrate, fat and protein metabolism was examined in the metabolic experiments.

2.3.3.1 Blood glucose level (BGL), glucose tolerance test (GTT)

Measurements of BGLs were performed before, during and after internationally standardized i.p. GTTs (D-glucose, 0.75 g/100 g bw/ml) both, in the acute and subacute phase (4 weeks after the intracerebral microinjection) of the experiments.

2.3.3.2 Plasma metabolite measurements

Relevant plasma metabolites (total cholesterol, HDL, LDL, triglycerides, uric acid) were determined by a ”cold chemistry” photometer (Arkray, Spotchem, Japan).

2.3.4 Behavioral experiments

The effect of intracerebral STZ treatment on taste perception and taste-associated aversive learning ability of rats was studied in two behavioral paradigms.

2.3.4.1 Conditioned taste aversion learning (CTA)

In advance to the intracerebral microinjection, the animals assigned for the CTA experiment learned to consume the daily amount of their water intake for 30 min from 10:00 to 10:30 a.m. every day. During the training period, rats were offered 0.1% sodium-saccharin solution (dissolved in tap water) several times to habituate the taste later serving as conditioned stimulus. On the pairing day, animals of both groups drank saccharin in the drinking period 30 min. prior to intraperitoneal injection of gastrointestinal malaise inducing LiCl (0.15 M, 2 ml/100 g bw). This conditioning procedure was followed on the next day by *ad lib*, then, on the next two days, by 30 min water drinking. On the test day, the saccharin solution was offered again exactly at the same time (from 10:00 to 10:30 a.m.). The consumptions of STZ treated and control animals measured on the pairing and the test day were statistically compared.

2.3.4.2 Taste reactivity

An adapted and modified, internationally accepted version of the protocol introduced by Grill and Norgren [94] was used in our laboratory. The animals were placed into a Plexiglass cylinder of 30 cm in diameter and 30 cm height. The behavior and taste responsiveness of the rats in the cylinder were videotaped. The cylinder was placed on a wooden frame mounted by a mirror in a 45 degree tilted angle. This enabled the easy observation of the animal's mouth movements throughout the whole testing period. The PE cannula, implanted into the oral cavity and fixed in the skull of the animal, was connected to a 1.25 m long PE tube (o.d. 3 mm), and this cannula was interconnected with the syringe placed in the infusion pump and filled by the appropriate gustatory stimulus solution. Taste stimuli represented the five basic tastes in two concentrations: sucrose 0.05 M, 0.5 M; NaCl 0.05 M, 0.5 M; MSG 0.05 M, 0.5 M; HCl 0.03 M, 0.3 M; QHCl 0.03 mM, 3.0 mM. The two well-defined, species specific response patterns (ingestive and aversive ones, including the mimical and postural-locomotor components) were evaluated and compared in the cytokine treated and control animals.

2.4 Histology

After electrophysiological and behavioral experiments, the location of the tip of electrodes or the sites of the microinjections were histologically verified in each rat. Data obtained in animals with mistargeted electrode or microcannula positioning, or with extensive brain tissue damage well beyond the limits of the NAcc, were excluded from further analysis.

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2.5 Data processing, statistics

In the statistical analysis of results of the electrophysiological experiments, Student *t*- and χ^2-tests were used. All data obtained in the behavioral and metabolic studies are expressed as means ± SEM. The SPSS for Windows software package was used for statistical analysis of experimental data. Analysis of variance (One-way ANOVA), the Tukey's test for post hoc comparisons, and the Student *t*-tests were employed. Differences were considered to be significant only at the level of $p<0.05$ or less.

3. Results

3.1 Electrophysiological experiments

By means of the tungsten wired multibarreled glass microelectrode, altogether the activity of 158 neurons was recorded in the rat (143) and monkey (15) NAcc. Since the electrophysiological characteristics of neurons proved to be essentially the same in the two species, and there was no significant difference between the anesthetized and the alert conditions, summed data were processed in the analysis.

3.1.1 Glucose-monitoring neurons in the NAcc

Glucose-monitoring neurons were identified by the multibarreled microelectrophoretic technique in the NAcc of both the anesthetized rats and the alert rhesus monkey. We examined

glucose-sensitivity altogether in case of 131 neurons, and out of them, 32 cells (24.4%) were proved to be GM neuron. It was established that the proportion of accumbens GM cells is considerably high, it is about 25% in both species.

Concerning the proportion and the type of these specific chemosensory neurons, their differential distribution in the two subdivisions of the rodent NAcc has been demonstrated. In the shell region 11 (19%) of 59 cells, while in the core 17 (29%) of 58 neurons showed responsiveness to glucose. In the shell 3 (27%) of the 11 GM neurons were proved to be GR, while 8 (73%) were GS cells. There was an opposite tendency in the core subdivision, 14 (82%) GR and 3 (18%) GS neurons were detected.

3.1.2 DA-sensitive neurons in the NAcc

More than half (54, 57%) of all examined neurons (94) in the NAcc were proved to be DA sensitive. In concordance with the determinant dopaminergic innervation of this limbic structure, in the shell region 26 (49%), in the core 28 (68%) neurons showed DA responsiveness. Interestingly, in the shell division of accumbens, the catecholamine had dominantly (in 89%) inhibitory effect, while DA induced excitation and suppression of activity in similar ratio (46% vs 54%, respectively) in the core subdivision.

3.1.3 Other neurotransmitter effects in the NAcc

We also studied the neuronal effects of two other “classical” neurotransmitters, GABA and Ach, in the Nacc. The responsiveness to GABA was examined altogether in case of 74 neurons (45 shell; 29 core). Of these cells, 35 (47%) changed in activity in response to microelectrophoretic administration of GABA. Exclusively inhibitory, never facilitatory, firing rate changes were seen to GABA. The GABA responsiveness of neurons in the two subdivisions was found remarkably different. While only 17 (38%) of the shell neurons showed GABA sensitivity, in the core 21 (74%) neurons of all cells tested changed in activity in response to GABA.

The Ach responsiveness of accumbens neurons was examined in case of 29 cells. The neuronal sensitivity to Ach was also found to be regionally different, effectiveness of the

neurotransmitter significantly differed in the two subregions of Nacc. In the shell, 7 of 15 cells, whereas in the core only 3 of 14 neurons changed their activity to the microelectrophoretic administration of Ach.

In our electrophysiological experiments, the effect of microelectrophoretically applied STZ was examined in case of 21 neurons. Six of them showed responsiveness to STZ. Regardless of recording either excitatory or inhibitory firing rate changes, the repeated administration of STZ always led to the irreversible termination of neuronal activity. All STZ sensitive neurons proved to be GM cells, at the same time, the STZ insensitive units were all GIS neurons.

3.1.4 Neuronal taste responsiveness in the NAcc

In addition to testing endogenous chemosensitivity (to neurotransmitters and modulators), the exogenous chemosensitivity (to gustatory stimuli) was also examined in 85 accumbens neurons. Out of the 85 cells, altogether 54 (64%) showed taste responsiveness. The proportion of taste sensitive GM units was 76% (16 of 21), but in case of GIS neurons it was only little more than 50%. The specific chemosensory (GM) neurons characteristically (12 of 16) changed their activity to two or more tastants, the GIS cells (31 of 38) only to one taste quality. Altogether 48 of the 54 taste-cells were also tested to DA responsiveness. DA sensitivity was proven in case of 33 (69%) of them. The proportion of DA sensitive gustatory cells (11) among the GM units was found to be the same.

We examined the effect of D1 or D2 receptor antagonists in case of 12 DA sensitive, taste responsive neurons. It was verified in 7 taste-cells that at least one of the DA blockers prevented the development of the gustatory response.

3.2 Metabolic and behavioral experiments

3.2.1 Change of blood glucose level in glucose tolerance test (GTT)

During the glucose tolerance test after the bilateral STZ microinjection into the Nacc pathological blood glucose levels were measured for a longer period of time, and the 18^{th} and 30^{th} min samples were in the diabetic range. In case of the STZ treated animals, not only the high

values of their blood glucose concentrations but the dynamics of changes were also different from those of the rats in the control group. The blood glucose level of control animals continuously decreased from the 18th minute, however, that of the rats with STZ microinjection into the accumbens increased till the 30th minute when it was significantly higher than the corresponding value in the control group.

In contrast to what was observed in the acute experiments, in the subacute phase (4 weeks after the intracerebral microinjection), there was no significant difference between the blood glucose concentrations and the dynamics of the curves of the control and STZ treated groups during the GTT.

3.2.2 Plasma metabolite alterations

Of the examined metabolites, there was no significant difference between the HDL, LDL and uric acid concentrations of the STZ treated and control groups. At the same time, however, the plasma triglyceride and total cholesterol levels were found significantly decreased in the STZ microinjected animals.

3.2.3 Conditioned taste aversion

The control rats drank significantly more of the saccharin solution on the conditioning day than on the test day, but in case of the STZ treated animals there was no significant difference between the saccharin consumption on these two days. Thus, STZ microinjection into the nucleus accumbens resulted in the failure of acquisition of CTA.

3.2.4 Taste reactivity

Although both the control and the STZ treated rats responded mainly with ingestive behavioral patterns to the hedonically pleasant taste stimuli, the animals with intracerebral STZ microinjection showed significantly less accepting reactions to these tastants than did rats of the control group. The difference between the rejecting behavioral patterns displayed to these pleasant taste stimuli just did not reach the level of significance, nevertheless, the frequency of

aversive patterns shown by the STZ treated rats was more than double than that of the control animals.

As expected, the control rats reacted with robust rejection to the unpleasant tastes, the STZ treated animals, however, displayed as many ingestive as aversive patterns. Furthermore, their behavioral patterns contained significantly more ingestive and significantly less aversive components compared to responses of the control group.

III. Discussion

1. ”Endogenous” chemosensory attributes /GR, GS/

The endogenous chemical responsiveness of neurons of a given brain region is highly determined by their neurotransmitters and modulators characterizing their input-output organization. The presence of many neurotransmitters and neuromodulators has already been verified in the NAcc: to our knowledge, GABA, Ach, DA, NA, serotonin, histamin, and opioid receptors are all present in this key structure of the forebrain limbic circuitry [86,87,95-101]. In the light of the above, it is not surprising that in case of all neurons tested by the multibarreled microelectrophoretic technique a certain degree of endogenous chemical sensitivity could be verified.

1.1 Glucose-monitoring neurons

We consider those results of our experiments especially important which provide clear evidence for the existence of GM neurons, intimately involved in the central regulation of feeding, in the nucleus acumbens of the rat and rhesus monkey. Although this kind of chemosensory neurons have already been identified in various other areas of the brain [1,30,32-35], this is the first time to verify their existence in this central region of the limbic system. When studying the location of GM neurons in the accumbens, it turned out that neural cells with differential responses to glucose show a characteristic topographic distribution in the rodent NAcc. The GS (inhibitory type) neurons were localized mainly in the shell, whereas the GR (facilitatory type) cells were predominantly identified in the core division. It is known that the

core plays an important role in the development and the guidance of conditioned behavioral forms. The excitotoxic lesion of this region led to the decrease of feeding and body weight [71,75,76,90]. At the same time, the shell region is reportedly involved in the control of non-conditioned behaviors and the selective inhibition or damage to it in rodents leads to increasing food intake and body weight [71,74-76,90,102,103]. The specific, differential distribution of the GM neurons in our study could be in causal relationship to the above distinct functional characteristics of the two subregions of accumbens. Recent data demonstrating the structural shell-core dichotomy of NAcc in the monkey and human brain further emphasize the general significance of the present results obtained in rodents [104].

1.2 DA sensitive neurons

The MLDS consists of fibers originating in A10 neurons in the VTA, A8 cells laterally surrounding the SN, and in the retrorubral neuron group [105]. One of the most important projection area of the MLDS is the NAcc [105-107]. In vivo microdialysis experiments provided evidence for that DA release in the Nacc is the essential condition and accompanying phenomenon of the development of reinforcement [69,70,108], and this fact was also confirmed by the blocking effect of DA antagonists injected into the NAcc [109,110]. Furthermore, it has also been demonstrated that DA injected locally into the accumbens, especially into the shell region, is itself rewarding, and that experimental animals could be conditioned for self-injection here by the DA reuptake inhibitor amphetamine [111,112].

The direct neuronal effect of DA in the NAcc was proved by our electrophysiological experiments. More than 50% of the examined neurons in this limbic stucture showed DA sensitivity. Approximately half of the neurons in the shell, whereas more than 2/3 of them in the core responded to the microiontophoretic application of DA. Our finding that there is a regional difference in the direction of predominant activity changes caused by DA further emphasizes the distinguished role of DA transmission in the NAcc.

1.3 GABA sensitive neurons

The 90% of all neurons in the NAcc are medium size spiny GABA-ergic cells [95]. It is known that inhibition of the shell region of NAcc induces robust food intake in the rat. This phenomenon is explained by the ceasing of indirect inhibition on the LHA which suppressive effect is otherwise exerted by GABA neurons located here [103]. We proved in our electrophysiological experiments both in the rat and rhesus monkey that approximately half of the NAcc neurons are inhibited by GABA. Based on observations in behavioral-neurochemical experiments, a differential GABA-ergic modulation [76] of the shell and core subdivisions has been reported recently. Our present data indicate that the neural basis of this functional dichotomy can be - at least partly - the notably distinct proportion of the GABA-sensitive neurons in these regions.

2. ”Exogenous” chemical sensitivity

As a specific motivation, the hunger appears at the beginning, appetitive phase of the feeding process, and the animal starts to search for food because of that. The initiative phase comes next, and this is controlled by the sight, smell and taste of the food, moreover, by the physiological changes caused by these sensory-perceptual factors. Gustation crucially determines the choice of foods and fluids, so that this is the most important external sensory determinant of the hedonic-motivational control during food intake.

2.1 Electrophysiological-neurochemical properties

The existence of taste responsive neurons in the NAcc was proven in our experiments. Our findings clearly showed that the proportion of GM neurons was remarkably higher among the gustatory cells compared to those which did not respond to tastes. It is worth noting that GM neurons of the NAcc displayed higher sensitivity to the aversive taste qualities. The GM taste-neurons responded characteristically to two or more taste stimuli, so these cells probably play an integrative role in the central feeding control by a common estimation of the relevant exogenous chemical stimuli. The GIS taste cells mostly responded to only one gustatory stimulus, indicating

that these neurons rather play a discriminative role in the central taste information processing. Postulated differential functions of the GM and GIS neurons in the taste information processing has already been demonstrated by results of our previous experiments performed in other forebrain structures [33,56,57].

The involvement of NAcc in hedonic-motivational regulation is well known, and it is also clear that the DA-ergic system plays an important role in these mechanisms. Related studies elucidated that dynamic, receptor mechanism dependent change of the DA neurotransmission in the accumbens is the indispensable condition and concomitant phenomenon of the reinforcing process [109,110,113-115]. Experimental findings provided evidence for that the DA level increases during reinforcement [69,70,108], at the same time, for that before the acquisition of conditioned taste aversion the unconditioned, whereas after pairing the conditioned stimulus causes decreasing levels of DA [116].

It was demonstrated in our present investigations that taste stimulation associated neuronal activity changes could be prevented or suspended by preceding or simultaneous microelectrophoretic administration of D1 and/or D2 receptor antagonist. These findings - in concordance with previous results of our research group [56,57] - substantiate our hypothesis concerning the determinant role of limbic forebrain DA neurotransmission in the central taste information processing.

2.2 Gustatory disturbances revealed in behavioral experiments

It is known from previous studies that both subregions of the NAcc take part in taste-associated motivational and learning mechanisms [72,73]. It was revealed in our conditioned taste aversion experiments that this strong adaptive function suffers deficit after the STZ microinjection induced selective destruction of GM neurons; this learned form of avoidance behavior does not develop in the animals of the STZ treated group. It is consequently supposed that the loss of GM cells of the NAcc leads to serious hedonic-motivational and consecutive taste information processing learning deficits. This hypothesis is also substantiated by our previously mentioned electrophysiological data.

The alterations observed in our taste reactivity tests elucidated the complexity of gustatory disturbances seen after STZ microinjection into the NAcc. Although the neurotoxin treated

animals also showed more ingestive than aversive patterns to the pleasant tastes, the behavioral acceptance to the ingestive tastes was still less pronounced in the STZ treated rats. Gustatory disturbances elicited by the STZ microinjection were even more obvious in case of behavioral responsiveness to the unpleasant tastes. The neurotoxin treated animals felt the aversive tastes significantly more pleasant than rats in the control group: they showed less aversive patterns, and there was no significant difference between their ingestive and aversive patterns displayed to the unpleasant tastes. These findings prove that though the ability of recognizing tastes is not totally lost by destruction of the GM cells in the NAcc, nevertheless, the hedonic-motivational estimation of exogenous chemical stimuli gets remarkably disturbed. It can be stated that after the STZ treatment the „perceptual monitoring" of tastes has got altered, so that difficulties occurred especially in the recognition of and adequate (adaptive) responding to the unpleasant tastes. This notion is also substantiated by our previous electrophysiological observations revealing a remarkably higher responsiveness of the GM neurons to the unpleasant than to the pleasant tastes.

The physiological significance of gustatory disturbances unraveled in our behavioral experiments can hardly be disputed. Consequences of STZ microinjection into the NAcc obviously influence the selection of foods since these adaptive processes help the animal to decide which foods - with characteristic taste - must be avioded. The recognition and distinction of tastes is of decisive importance at this first step of processes involved in self-maintenance. If there is an abnormal sensation already at this level, then, the adequate behavioral form does not work effectively; on the one hand, the animal could consume harmful, poisonous "food" objects, on the other hand, it could reject a favourable, high energy content food, this way becoming handicapped compared to its healthy conspecific mates.

The involvement of several brain structures (OBF, AMY, LHA, GP) has already been proven in the central taste information processing [38,55,56,117-121]. It is well known, that the NAcc is tightly interconnected with these structures [77-79], and the data of literature verify that the NAcc itself plays direct role in the central control of gustatory functions [73,122-128]. This notion, on the one hand, is confirmed by findings of our behavioral and electrophysiological experiments, on the other hand, it was even further elaborated especially with regard to elucidating the complex, integrative role of accumbens GM neurons.

3. Metabolic alterations

According to our present results selective destruction of GM neurons in the nucleus accumbens leads to multiple metabolic changes. Behavioral-metabolic experiments of our laboratory previously demonstrated that STZ microinjection into the VMH or OBF causes metabolic deficits similar to those seen in type 2 diabetes mellitus [34].

The STZ treatment of NAcc resulted in similar consequences to the above. In addition to the pathological alteration of carbohydrate metabolism revealed in glucose tolerance test, we also demonstrated the disturbance of fat metabolism (with decreasing level of total cholesterol and triglycerides), and the elevation of plasma uric acid concentration as well. This latter change is in harmony not only with our previous results [34] but with the activation of proteolytic processes experienced in diabetes mellitus, and also with data of literature concerning the consequent hyperuricemia in these pathological conditions [129,130]. Our dyslipidemic results well fit in the line of previous observations that showed the alterations of the concentration of cholesterol and even more of those of the lipoprotein fractions (VLDL, LDL, HDL) in both types of DM [131-133].

The differences of these symptoms after interventions in various brain regions, on the one hand, refer to the obviously differential roles of these structures - VMH, OBF, NAcc - in the regulation of metabolism. On the other hand, the striking fact should also be emphasized that peripheral metabolic changes take place very quickly, necessarily due to neural mechanisms induced by the manipulation of a specific group of neurons in a circumscribed brain region. The direction and the dynamics of these alterations are probably influenced by many factors, among others the actual plasma insulin and the leptin concentrations [134], therefore, the extension of metabolic measurements in this direction is indispensable in our further experiments. Nevertheless, it has been clearly proven that destruction of the forebrain GM neurons causes major alterations of the carbohydrate, fat and protein metabolism, in several aspects similar to those seen in type 2 DM.

The present findings, along with our results on complex functional attributes of the GM neurons and on the perceptual-motivational changes seen after STZ microinjection, all support our hypothesis that these specific neurons play essential role in the preservation of homeostatic balance in the healthy organism. If this is true, it is reasonable to suppose that the damage to

these chemosensory cells because of any reason will necessarily disturb the balance of homeostasis that further on can elicit and maintain feeding and metabolic illnesses.

IV. General conclusions

Nowadays, because of their increasing public health significance, the studying of processes in the background of feeding and metabolic disorders becomes more and more important. The obesity, the diabetes mellitus and the metabolic syndrome are already considered widespread ”epidemias” involving major part of the population. In developed countries, more than 25 percent of diseases - many with extreme mortality - are of, at least partially, metabolic ethiology. The present, generally accepted medical-clinical view put in the focus the peripheral processes and pathological deviations during establishing the diagnosis and designing the therapy of these illnesses. At the same time, however, a central origin or CNS involvement is more and more suggested or even proven in case of the above diseases. Our investigations provided evidence for that the damage or destruction of the GM neurons in the NAcc lead to an internal imbalance of the organism. The better understanding of NAcc and other brain structures important in the maintenance of homeostasis, and that of the functioning of their specific chemosensory neurons should be of outstanding significance with respect to recognizing the pathological processes in the background of alimentary and metabolic diseases. This could subsequently lead to more effective prevention and development of successful therapeutic strategies of these diseases.

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Own publications related to the thesis

I. Papers:

1. Karádi, Z., B. Lukáts, Sz. Papp, Cs. Szalay, R. Egyed, L. Lénárd, and G. Takács: Involvement of forebrain glucose-monitoring neurons in taste information processing: Electrophysiological and behavioral studies. Chemical Senses, 30:168-169, 2005.

2. Papp, Sz., B. Lukáts, G. Takács, Cs. Szalay, Z. Karádi: Glucose-monitoring neurons in the nucleus accumbens. NeuroReport , 18(15):1561-1565, 2007.

II. Books, chapters, proceedings:

1. Karádi, Z., B. Lukáts, Sz. Papp, G. Takács, R. Egyed, and L. Lénárd: The central glucose-monitoring neural network: major protector of the adaptive homeostatic balance for well being of the organism. In: Brain-Inspired IT I, (Eds. H. Nakagawa, K. Ishii and H. Miyamoto), International Congress Series Vol. 1269, Elsevier, Amsterdam, pp.30-33, 2004.

III. Abstracts:

1. Papp, Sz., Lukáts, B., Szalay, Cs., Göde, J., Hernádi, I., Kellényi, L. and Karádi, Z.: Taste-responsive neurons in the nucleus accumbens of the rat. Neurobiology 9(3-4): P. 347-348, 2002.

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