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Action of cholecystokinin and serotonin on lateral hypothalamic neurons of rats

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Abstract

Discharges of spontaneously active lateral hypothalamic neurons were extracellularly recorded during iontophoretic administration of cholecystokinin (CCK-8S) or/and serotonin (5-HT) in anesthetized rats. The main results are the following. (1) The proportion of neurons responsive to CCK-8S was 62% (61/99) and that responsive to 5-HT 42% (33/78). (2) Out of the neuronal sample, 36% were influenced by both transmitters, allowing an interaction between the two systems. (3) Co-ejection of CCK and 5-HT elicited a response in 40% of the tested neurons, which was a significantly smaller responsiveness than with separate ejection of CCK-8S. The effect resulted from a reduced number of excited neurons whereas the number of inhibitions did not change. The results show that effects of 5-HT and CCK can converge on the same neuron within the lateral hypothalamus. This might be of relevance in the regulation of feeding behavior.

Keywords: Cholecystokinin; 5-HT (5-hydroxytryptamine, serotonin); Hypothalamus, lateral; Single-unit activity; Microiontophoresis; Urethane

1. Introduction

The hypothalamus is involved in many different functions. One of these is the regulation of food intake and body weight. As early as 5 decades ago, it was found that electrical stimulation of the lateral hypothalamic area or destruction of the ventromedial hypothalamus results in hyperphagia, whereas lesioning the former or stimulating the latter induces anorexia (Schick et al., 1994). The mechanisms by which the lateral hypothalamus is integrated in the regulatory network of nutrition are not yet clear.

A significant amount of evidence has revealed that a diversity of transmitters and gut hormones can modulate feeding at peripheral as well as central sites. Among these, cholecystokinin (CCK) and serotonin (5-HT) were found to play an important role (reviewed in: Blundell, 1984; Silver and Morley, 1991; Schick et al., 1994; Bernardis and Bellinger, 1996). Loading the stomach with a test meal or water was followed by an increase of CCK within the lateral hypothalamus, and microinjection of CCK locally into this region or intracerebroventricularly influences feeding termination and satiety (Schick et al., 1994). The effect of 5-HT on feeding behavior resembles in many respects that of CCK. During feeding, an increase in the content of 5-HT in the lateral hypothalamus has been found (Schwartz et al., 1989), and manipulations of extracellular 5-HT in the lateral hypothalamus influenced food intake (Blundell, 1986). Therefore, an interrelationship is to be expected between both mediators within this hypothalamic structure. Although there are a few studies on the influence of CCK (Shiraishi, 1990) or 5-HT (Kai et al., 1988) on the activity of lateral hypothalamic neurons, an investigation of the effects of both transmitters on one and the same neuron does not exist.

The purpose of the present work was to evaluate and compare the responsiveness of lateral hypothalamic neurons to locally administered CCK and 5-HT, and to investigate the interaction of CCK and 5-HT at the neuronal level using separate administration as well as co-administration of both drugs. Due to the mentioned behavioral effects of CCK and 5-HT in the lateral hypothalamic area, it was assumed that its neurons respond to both transmitters. Co-ejection of both drugs should evoke additive effects or perhaps potentiation.

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2. Materials and methods

Experiments were carried out on adult male and female Wistar rats. They lived in an animal house with 12-h light and dark cycle, room temperature of 21–23°C and free access to standard rat chow pellets and tap water.

Each experiment started in the morning with an overnight food-deprived animal. The rats were anesthetized with urethane (initial dose 1.2 g/kg i.p., subsequent injections as needed, for details, see Albrecht and Davidowa, 1989) and placed in a stereotaxic instrument. A feedback-controlled heating pad maintained the rectal temperature at 37 ± 0.5°C. A hole was drilled into the skull to insert the electrode toward the lateral hypothalamus (co-ordinates 3.0 mm behind bregma, 2.5 mm lateral to the midline, depth from the surface 7–9 mm). All procedures were carried out in accordance with the European Communities Council Directive (1986) and proved by the Regional Animal Ethics Committee (G 0315/95) in Berlin.

Recording techniques and equipment were the same as described previously (Albrecht et al., 1993). Glass micropipettes for extracellular recording were filled with saturated solution of trypan blue (DC resistance 10–30 MΩ) for iontophoretic marking of the recording site. The electrode was glued to a seven-barrel pipette with its tip extending about 30 μm beyond the pipette tip. Each barrel was filled with one of the following chemicals: CCK octapeptide sulfated (CCK-8S), the selective CCK_A receptor antagonist [*S*-(*R**,*S**)]-β-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[tricyclo[3.3.1.1.^{3,7}]dec-2-yloxy)carbonyl]amino] propyl]amino]-benzenebutanoic acid *N*-methyl-D-glucamine (PD 140.548), the selective CCK_B receptor antagonist (4-{[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2[[[1.7.7.-trimethyl-bicyclo[2.2.1]hept-2-yl-oxy]carbonyl]amino]-propyl)amino)-1-phenylethyl]amino-4-oxo-[1*S*-1α.2β[*S**-(*S**)]4α]}-butanoate *N*-methyl-D-glucamine (PD135.158) all dissolved in phosphate buffered saline (0.25 mM, pH 7.8 each), 5-hydroxytryptamine creatine sulfate (5-HT, serotonin, 20 mM, pH 4.5), the selective 5-HT_{1A} receptor agonist (±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (8-OH-DPAT 15 mM, pH 4.5), and the selective $5\text{-HT}_2/5\text{-HT}_{1C}$ receptor agonist ((±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI, 10 mM, pH 4.5), all from Research Biochemicals International (RBI, Natick, USA). The last channel was filled with NaCl (165 mM) and used as balance electrode and for checking current effects. The drugs were ejected with currents between 30 and 90 nA, in general 50 nA, and retained with 3–5 nA (Ionophor 3, Biologic). The ejection time was mostly 2 min.

The procedure for each neuron is the following: (1) application of CCK-8S and 5-HT separately, to analyze the responsiveness to these drugs, (2) co-administration of both transmitters in order to investigate their interactions, (3) co-administration of CCK-8S with each of the two antagonists to establish the involved CCK receptor type and (4) ejection of the selective 5-HT receptor agonists to obtain hints of the serotonin receptors in the lateral hypothalamus. The time between the individual applications had to be prolonged until a full recovery was reached, requiring between 5 and 30 min. Finally, the recording site was marked by microiontophoretic ejection of trypan blue (10 min, 10 μA negative current).

Extracellularly recorded electrical activity was amplified and displayed on an oscilloscope. Each record contained only one type of large spikes which were discriminated using a window discriminator (World Precision Instruments) and transformed into standard pulses. These were then fed through an interface (CED 1401, Cambridge Electronic Design) to a personal computer, stored on disc and used for computing frequency–time histograms (Spike 2 software) which were displayed on-line during sampling. Off-line, we then selected the 1-min time interval during or following a drug ejection with the strongest alteration of the impulse frequency to compute the number of spikes within this minute and to compare it with the data of the last minute before onset of drug application. If the impulse rate changed by at least 30% (or 0.5 impulses/s in neurons firing less than 1 impulse/s) this was regarded as response to the drug administered. In the (seldom seen) case of biphasic changes of the activity the first direction that reached the criterion was taken. An analysis only during the time of drug application or with a fixed time delay seemed to be unsuitable. First, the substances used hardly induce fast effects as Glutamate or $GABA_A$ receptor agonists. A variable delay in the effect also can be expected due to the difference in the distance between the tip of the electrode and the investigated neuron.

The number of the responses was counted and compared by means of the χ^2-test. Additionally, the impulse frequency elicited by drug application was compared to the pre-drug impulse frequency for an entire neuronal sample by the Wilcoxon matched-pairs signed-ranks test. This test can only reach a significance level if most of the included neurons change the activity in the same direction, e.g., increase or decrease. It illustrates therefore relatively uniform influences of a drug on the neuronal sample.

At the end of an experiment, the animal was killed by an overdose of urethane, decapitated and the brain removed and fixed in 10% formaldehyde. The localization of the blue spots was determined histologically in the frozen frontal brain slices.

3. Results

In this study the activity of 100 neurons located in the lateral hypothalamus was investigated, 41 were recorded in 21 males and 59 in 32 females (1.89 neurons/animal). It can be assumed that this sample represents a morphologically homogenous population, because neurons were described as being remarkably uniform with extraordinarily long dendrites and axons (Bernardis and Bellinger, 1993).

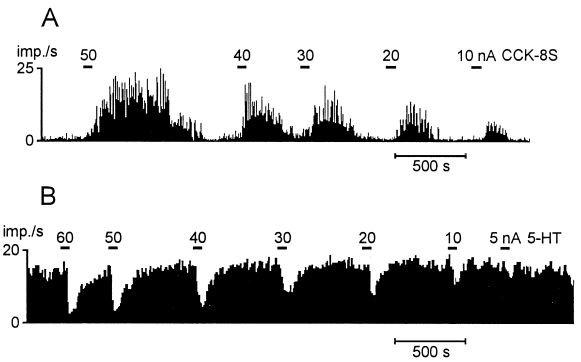

Fig. 1. Frequency–time histograms of two neurons located within the lateral hypothalamus. (A) CCK-8S dose-dependently increased the firing rate. (B) 5-HT dose-dependently decreased the firing rate. The bars above the histograms represent time and duration of iontophoretic ejection, the numbers show current intensity.

3.1. Responses to CCK-8S

CCK-8S was administered iontophoretically to 99 neurons. The peptide induced dose-dependent responses in 61 neurons (Fig. 1A). The number of excited neurons exceeded significantly that of inhibited cells (72 vs. 28%, $P < 0.001$, χ^2-test). Although one quarter of the responsive neurons was inhibited, the predominant effect following the CCK-8S ejection in the whole group ($n = 99$) was an increase in firing ($P < 0.001$, Wilcoxon test). The activity of neurons from males was more frequently increased by CCK-8S than that from females (56 and 36%; $P < 0.05$, χ^2-test), whereas the percentage of inhibitions did not differ (Fig. 2).

The co-administration of CCK-8S together with one or both selective CCK receptor antagonists was investigated in 12 CCK sensitive neurons. The CCK_A receptor antagonist, PD 140.548, blocked the response to CCK-8S in five neurons, whereas the CCK_B receptor antagonist, PD 135.158, antagonized the CCK-8S-induced effects in six neurons. In two neurons out of nine tested with each of the

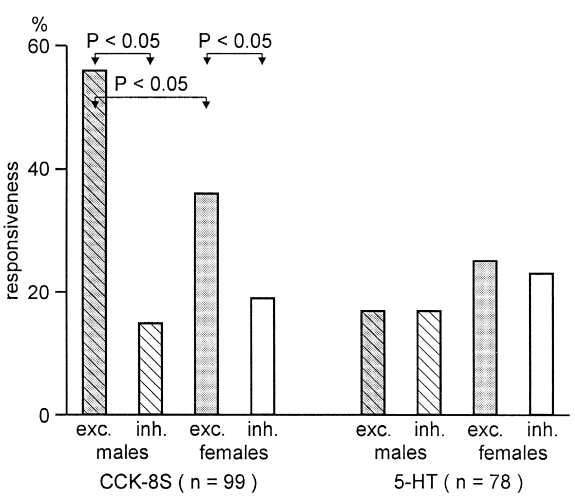

Fig. 2. Percentage of lateral hypothalamic neurons increasing (gray columns) or decreasing (white columns) their discharge frequency by more than 30% during iontophoresis of CCK-8S (left) or 5-HT (right). The samples were divided concerning the sex of the animals (males hatched columns, females blank columns).

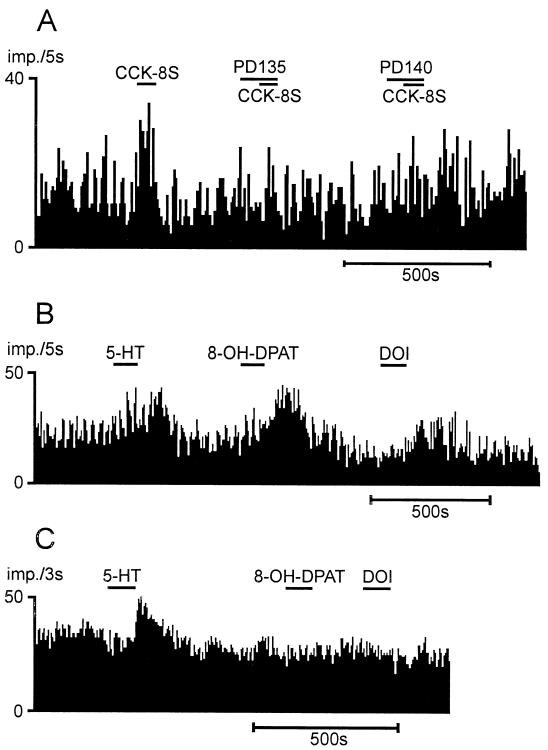

Fig. 3. Frequency–time histograms of the activity of three lateral hypothalamic neurons during the ejection of drugs (marked by bars). (A) The increased firing elicited by CCK-8S was partly blocked by both the CCK_B receptor antagonist, PD135.158, and the CCK_A receptor antagonist, PD140.548. (B) The neuron increased the firing to iontophoresis of 5-HT as well as to the selective 5-HT receptor agonists, 8-OH-DPAT and DOI. (C) This neuron was excited by 5-HT (the small decrease of the activity during 5-HT iontophoresis did not reach the criterion of 30% modification of the impulse rate) but not influenced by 8-OH-DPAT or DOI. Numbers in parenthesis: ejection current in nA.

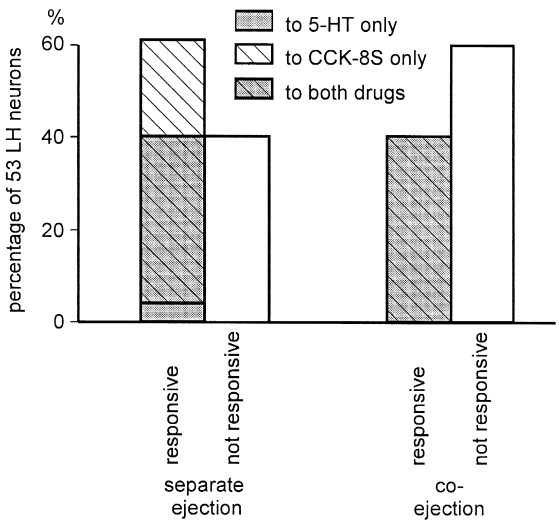

Fig. 4. Responsiveness of lateral hypothalamic neurons to separate ejection or co-ejection of CCK-8S and 5-HT. In the case of separate ejection (left columns) the percentage of neurons responsive to CCK-8S, to 5-HT or to both drugs was summed.

two drugs, the CCK-8S-induced responses could be partly blocked by both antagonists (Fig. 3A).

3.2. Responses to 5-HT

The influence of iontophoretically ejected 5-HT on the discharge frequency was tested in 78 neurons. The effects in responsive neurons depended on the amount of the ejection current (Fig. 1B). With 5-HT, 33 neurons responded (42%), which is a significantly smaller portion of neurons than in the CCK-8S sample ($P < 0.05$, χ_2-test). In contrast to the results obtained with CCK-8S, neurons in the male group showed lower responsiveness to 5-HT in comparison to the females (33 vs. 48%, $P > 0.05$, χ^2-test). The percentage of excited neurons resembles that of inhibited neurons in the male and female subgroups (Fig. 2). Therefore, the mean change of the impulse rate evoked within each group by the 5-HT iontophoresis was not significant ($P > 0.05$, Wilcoxon test).

In addition, the influence of selective serotonin receptor agonists on the neuronal activity was tested. Of 43 neurons, 15 were responsive to the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, and 11 out of 32 to the selective 5-HT_2 receptor agonist, DOI (Fig. 3B). Four 5-HT-sensitive neurons were found which showed opposite or no response to 8-OH-DPAT as well as to DOI (Fig. 3C).

Counting the number of neurons responding to 5-HT as well as CCK-8S ($n = 77$), we found 36% responsive to both drugs and 29% to one drug. Thirty-five percent of the neurons neither responded to CCK-8S nor to 5-HT. In males, all neurons influenced by 5-HT responded also to CCK-8S.

3.3. Interactions between CCK-8S and 5-HT

Co-administration of CCK-8S and 5-HT was performed in 53 out of 77 neurons. Of these cells 13 increased and eight decreased their firing rates by more than 30% (responsiveness 40%). The sample tested with co-administration of CCK-8S and 5-HT ($n = 53$) responded to separate CCK administration with 23 excitations and seven inhibitions (57% responsive neurons); ejection of 5-HT evoked an increase in the firing frequency in 12 neurons and a decrease in eight neurons (40% responsive neurons). Thus, the responsiveness to co-administration of both drugs did not increase but decreased compared to the effects induced by CCK-8S ($P < 0.05$, χ^2-test, one-tailed, Fig. 4). This reduction in responsiveness concerns only the excitatory effects of CCK-8S.

Comparing the observed changes in the discharge rate of individual cells elicited by co-ejection with those elicited with single ejection of CCK-8S or 5-HT, we found that, in most neurons, the drugs induced an additive effect (Fig. 5A), although in some neurons a potentiation seems to occur (Fig. 5B).

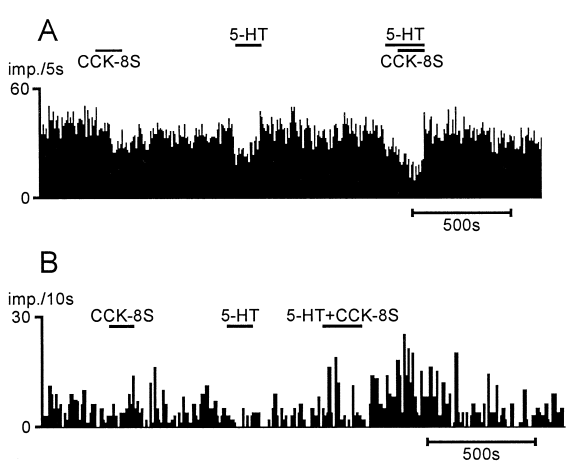

Fig. 5. Frequency–time histograms of the firing of two lateral hypothalamic neurons demonstrating the effects of iontophoretically administered CCK-8S, 5-HT and co-ejection of both. Time of iontophoresis of drugs marked by bars. Ejection current: 50 nA. The neuron in the upper diagram shows an additive effect of both drugs, while that in the lower histogram shows a potentiation of the CCK response.

4. Discussion

In the present study of the lateral hypothalamic area, we found a considerable proportion of spontaneously active neurons responsive to iontophoretically administered CCK-8S (62%) or 5-HT (42%). This result is in general agreement with earlier findings of Kai et al. (1988) and Shiraishi (1990), though there are differences in the details.

Concerning CCK-8S, Shiraishi found 33% of the unselected neuronal sample responsive. This difference might be attributed to the fact that the rats were satiated and therefore the lateral hypothalamic neurons could be less sensitive to exogenous CCK. Further, Shiraishi found nearly exclusively a suppression of activity, whereas in our sample, by far, more excitations were elicited. Most of the neurons of our sample showed relatively low discharge rates. In this situation, an inhibition of the activity is difficult to prove by extracellular recording. In contrast, Shiraishi drove the neurons by iontophoretically ejected 2-deoxy-glucose and tested the CCK effect on this background. Another cause for the observed differences may be a different location of the investigated neurons within the lateral hypothalamus, because its subregions have been found to be differently connected to other brain structures and may therefore be functionally incongruent (Veening et al., 1987; Aou et al., 1991). In addition, the different anesthesia used (chloralose/urethane mixture) should be taken into account. In other brain structures excitation was described as the main neuronal response to CCK-8S, although inhibition was also found (Boden et al., 1991; Albrecht et al., 1993, 1994; Davidowa et al., 1995; Zippel and Henklein, 1995).

Concerning the 5-HT effect, in the sample of Kai et al. (1988), 73% of the neurons reduced their firing and 5% were activated. We found a much smaller responsiveness

(38%) and slightly more facilitated than inhibited neurons. It is to mention that Kai et al. (1988) regarded 20% change in firing as responses in contrast to 30% in our sample. Furthermore, here again the motivational state and the anesthesia differ and the sample of Kai et al. (1988) only included glutamate sensitive neurons. Aoyagi et al. (1992), using freely behaving rats, showed parallel to feeding behavior an increased 5-HT turnover in the lateral hypothalamus on the one hand, and on the other, in a sample of 30 neurons of this region an increased activity in 12 and a decreased firing in seven cells. The alteration in neuronal activity may be related to the activation of the serotonin system induced by food intake. Also excitation and inhibition were similarly frequent, as it was found in our sample.

No study seems to exist comparing the effect of both transmitters on the same lateral hypothalamic neuronal sample. Knowledge to this point may be important on the background of the similarity of the effects which they can elicit in the context of feeding. An interaction of these two transmitter systems concerning the regulation of ingestive behavior has been proposed already for nearly 1 decade. Cooper and Dourish (1990) have proposed an interactive model suggesting that both the elevated CCK activity and the elevated 5-HT activity were involved in a logical AND gate in determining the onset of satiety. It could be speculated that the lateral hypothalamus may function as such an integrative structure. We found one-third of the tested neuronal population responsive to both transmitters. In this pool, a direct integration of the information mediated by both systems can take place, although effects below the level of a change of discharge rates have to be taken into account.

In addition, Cooper and Dourish (1990) proposed that the two transmitter systems should promote each other. Our results obtained from the lateral hypothalamic neuronal sample do not support this last assumption. The number of responsive neurons did not increase during the co-administration of the two transmitters. On the contrary, the number of excitatory effects of CCK-8S was reduced. potentiation of the effect of one transmitter by co-ejecting the second one is seldom found. Most neurons seem to produce either an additive or subtractive effect, respectively.

Concerning the CCK receptors involved in the mediation of the effects, we were able to demonstrate an influence via CCK_A as well as CCK_B receptors, partly on the same neuron. Both types of receptors may be expressed by the neuron investigated. But it cannot be excluded that receptors located presynaptically or on nearby neurons may be involved, although local circuit neurons were not found in the lateral hypothalamus (Millhouse, 1979).

In binding studies, CCK receptors were only found in other hypothalamic nuclei, but not in its lateral area (Hill et al., 1992). However, behavioral effects elicited by CCK injection into the lateral hypothalamus make likely the existence of CCK receptors in this region. Although CCK_B receptors are common in the brain, CCK_A receptors are only found in restricted areas (Crawley and Corwin, 1994). Functional studies demonstrated CCK_A receptors outside these originally defined areas (Albrecht et al., 1994; Davidowa et al., 1997; Voigt et al., 1998). Hill et al. (1992) have supposed that CCK_A receptors should be clearly more widespread in distribution in the brain than earlier suggested.

The 5-HT effects found in our investigation seem to be mediated by $5\text{-}HT_{1A}$ and $5\text{-}HT_2$ receptors, because the selective agonists 8-OH-DPAT and DOI, both were effective to evoke responses. Both types of receptors are known to exist in the hypothalamus (Jhanwar-Uniyal et al., 1994; Waeber and Moskowitz, 1995; Samanin and Grignaschi, 1996; McQueen et al., 1997). Although $5\text{-}HT_{1A}$ receptors have been shown to be present especially on presynaptic sites (Maswood et al., 1995), it has been demonstrated that postsynaptic 1A type 5-HT receptors are also involved in the regulation of food intake (Jhanwar-Uniyal et al., 1994). Some neurons in our sample seem to be influenced via both types of 5-HT receptors. Because there were neurons responding to 5-HT but not to 8-OH DPAT nor to DOI, the possibility should be taken into account that further types of 5-HT receptors are present in the lateral hypothalamus. Recently, the presence of $5\text{-}HT_{1B}$, $5\text{-}HT_{1D}$ (Waeber and Moskowitz, 1995), and $5\text{-}HT_7$ receptors (Gustafson et al., 1996) has been reported in the hypothalamus. Alternatively, it could be assumed that the amount of ejected drug was insufficient to influence the firing of these neurons in the case of 8-OH DPAT and DOI.

The different percentage of CCK responsive neurons in males and females may be related to sex differences in body weight regulation, which have been described earlier. A sex-dependent satiety effect of CCK has been demonstrated (Voits et al., 1996). Loss of body weight after electrolytic lesion of the lateral hypothalamus has been found to be more severe in males than in females, whereas hyperphagia after lesion of the ventromedial hypothalamus developed mainly in females (Cox and Kakolewski, 1970). Neurotoxic lesions of these nuclei have been effective to produce changes in body weight even in males (lateral hypothalamus) or females (ventromedial hypothalamus) only (Lenard et al., 1991). These differences may be mediated by sex hormones. Testosterone, for example, has been found to modify the neuronal activity of the lateral hypothalamus of male rats (Orsini et al., 1985) and estradiol has been shown to influence the feeding related CCK (Geary et al., 1996) and 5-HT (Maswood et al., 1995; McQueen et al., 1997) transmitter systems. Our results are in agreement with these earlier findings and support the hypothesis that the lateral hypothalamus may be more important for the regulation of food intake in males than in females.

In summary, the results show that the lateral hypothalamus can function as a site of integration of centrally acting

CCK and 5-HT. The elicited effects may be involved in the regulation of food intake.

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