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ORIGINAL ARTICLE

Action of prolactin, prolactin-releasing peptide and orexins on hypothalamic neurons of adult, early postnatally overfed rats

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Abstract

OBJECTIVES: Hypothalamic neurons of rats overweight due to early postnatal overfeeding (SL) differ from those of control rats in their responses to feeding relevant hormones like leptin or insulin. The question arose whether prolactin and prolactin-releasing peptide (PrRP) express also differential action in SL rats. These peptides are described to have an effect on food intake and body weight regulation. Prolactin is co-synthesized in lateral hypothalamic neurons together with orexins that were also analyzed in this study.

METHODS: Single unit activity was extracellularly recorded in brain slices from adult control rats (CL) and from rats previously raised in small litters (SL). The action of the peptides on the firing rates was evaluated in the medial parvicellular part of the paraventricular nucleus (PaMP) and the medial arcuate nucleus (ArcM).

RESULTS: In control rats, PrRP significantly activated PaMP neurons, whereas prolactin and orexin-A induced also inhibition. In SL rats, there was a significantly different effect of orexin-B on PaMP neurons: the main effect changed from activation in controls to inhibition. ArcM neurons of both control and SL rats were mainly excited by prolactin and orexins.

CONCLUSION: Changes acquired during early development of neuronal responses to feeding relevant peptides are not a general non-specific mechanism of neurochemical plasticity, but concern specific hypothalamic nuclei and/ or hormones and neuropeptides. The increase in inhibition by orexin-B of hypothalamic paraventricular neurons could in vivo contribute to the neonatally acquired disposition towards persistingly increased food intake and reduced energy expenditure of overweight SL rats.

Abbreviations:

ACSF	artificial cerebrospinal fluid
AGRP	agouti related peptide
ANOVA	analysis of variance
ArcM	arcuate nucleus, medial part
CL	control litter
CRF	corticotropin-releasing factor
DMH	dorsomedial hypothalamic nucleus
GABA	γ-aminobutyric acid
mRNA	messenger ribonucleic acid
NPY	neuropeptide Y
PVN	paraventricular nucleus
PaMP	paraventricular nucleus, medial parvicellular part
POMC	proopiomelanocortin
PRL	prolactin
PrRP	prolactin-releasing peptide
SD	standard deviation
SL	small litter
SNK	Student-Newman-Keuls test

Introduction

Compared to hypothalamic neurons of control rats, those of early postnatally overfed rats that maintain overweight disposition into adulthood have shown a different response to feeding relevant hormones and neuropeptides like the anorectic circulating satiety signals leptin and insulin, but also to the orexigenic neuropeptide Y (NPY) and agouti related peptide (AGRP) [5,6,7]. These differences in neuronal activity are regarded as a sign of developmental plasticity that leads to lasting malprogramming of regulatory processes of body weight [10,20,26,35]. Rats raised in small litters are hyperphagic and overweight throughout live and express hyperleptinaemia [7,35,36]. The plastic neuronal changes, however, are not necessarily a general phenomenon, but may selectively involve specific peptidergic systems. We aimed therefore to extend our studies on further peptides that are thought to play some role in body weight regulation.

Besides its known hormonal effect, prolactin (PRL) has an influence on the regulation of body weight. It has been shown to induce increased food intake, at least in female rats and ring doves [42,46]. Interestingly, it is synthesized in neurons of the lateral hypothalamus as co-peptide of orexins/ hypocretins [39] that are known for their orexigenic action, but are also involved in sleep control [48]. Prolactin receptors were observed in various hypothalamic nuclei, e.g., the paraventricular nucleus (PVN) and the arcuate nucleus [1]. PRL plays a special role in the regulation of food intake during lactation, but it seems to have effects also in male rats [13,27] that express fewer receptors in the hypothalamus than females [34].

Prolactin-releasing peptide (PrRP) is a 31 amino acid peptide [19] that has been found to occur in the hypothalamus only in discrete regions [30,40]. Beside its role in the release of prolactin and further functions [47] it seems to be directly involved in the regulation of food intake and body weight [23,24]. In the hypothalamus, the peptide is produced especially in the dorsomedial nucleus (DMH) [30,40]. Many fibres showing PrRP immunoreactivity have been detected in the PVN [30]. Surprisingly, there seem to be some antagonistic effects to prolactin concerning the regulation of food intake. PrRP may reduce food intake and increases energy expenditure [23,24,43] .

Orexins [41] or hypocretins [9] have been known since 1998 like PrRP [19]. Administration of orexins induces prolonged food intake [32,41] and increases arousal [48]. Missing of orexins is followed by narcolepsy and hypophagia, but also obesity [15]. There are various reports concerning their action on neuronal firing [9,17,38,44,45,51].

Materials and methods

All the experiments were performed in the offspring of an outbred colony strain of Wistar rats (Charles River Laboratories, Sulzfeld, Germany). To induce early postnatal overnutrition, the primary litter size was reduced on the third day of life to only three pups per litter (small litters, SL). In control litters (CL), the size was adjusted to 12 neonates per mother. The dams were singly housed during pregnancy and with the litter during lactation until weaning. They had free access to tap water and pellet diet (commercial control diet for rats; Altromin, Lage, Germany; Code 1314, energy content: 12.5 kJ/g, respectively Code 1326, 11.9 kJ/g, after the fourth week of age). The animals were kept under standard conditions with the normal 12-h light: 12-h dark cycle. Adult males of each litter size in an age between 70 and 120 days were randomly assigned to the electrophysiological studies presented here.

All procedures were carried out in accordance with the guidelines for the care of animals [12] and approved by the local Animal Care and Use Committee (G 0028-96 and T 139-99).

The rats were decapitated under ether anaesthesia. Coronal slices (350 or 400 μm) were cut with a vibroslicer from the brains submersed in ice-cold oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF, containing (in mM): NaCl 129, KCl 3, $NaHCO_3$ 21, $CaCl_2$ 1.6, $MgSO_4$ 1.8, NaH_2PO_4 1.25, glucose 10; pH 7.4). The slices were placed into an interface chamber for storage. They were continuously perfused with oxygenated ACSF (2 ml/min) at 35 ± 0.1°C. The electrophysiological studies were performed in a second chamber after equilibration of approximately 2 h. Action potentials of spontaneously firing neurons were extracellularly recorded with ACSF-filled glass microelectrodes (8-20 MOhm) from the medial parvicellular part of the paraventricular nucleus (PaMP) and the medial part of the arcuate nucleus (ArcM) [33]. The fornix and the 3rd ventricle served as landmarks for introduction of the electrode. They were used together with the mammillothalamic tract and the optic tract for determination of the coronal section (Bregma –1.8 mm until –3.6 mm [33]). Only neurons with regular baseline activity for at least five minutes were studied, whereas units that ceased firing shortly after detection were excluded from further investigations.

Stored as frozen aliquots of concentrated stock solutions, prolactin (recombinant, human, expressed in E. coli, Product L 4021), prolactin-releasing peptide (1–31), orexin-A and orexin-B (rat), purchased from

Bachem, Heidelberg, Germany, were freshly dissolved in warmed ACSF before administration in drops of 50 µl upstream to the slice directly into the experimental interface chamber. The peptides were used in a concentration of 10 nM. They were further diluted about 40 times by the perfusion medium (chamber volume approximately 250 µl) before reaching the examined neuron. Such doses were shown to have behavioural effects [23,32,41]. Only one neuron per slice was studied.

The data are presented as means ± SD. We used the paired t-test for determination of a significant activity change within a neuronal population, and ANOVA with following Student-Newman-Keuls test (SNK) for determination of differences between groups. We evaluated spontaneous variation of firing and the neuronal responses by counting the spikes/s of representative periods 100 s or 200 s before (twice) and under the influence of each drug. The effects were determined by calculating the difference between the rate of baseline firing and the drug-induced firing. For individual neurons, a change in the discharge rate by at least 20% was considered to be a response. For neurons discharging in rates below 1.2 spikes/s, a difference of 0.4 spikes/s was considered a response. These values exceeded those of spontaneous variation in firing. The proportions of responsive neurons in both groups were compared using the Chi^2-test. Statistical significance was accepted at the 95% confidence level ($p < 0.05$).

Results

The data derive from studies on brains of 70 normal CL rats and 64 overweight SL rats which were also used for other studies, e.g. [8]. The mean body mass did not significantly differ between the groups on day 3 of life (7.7 ± 1.3 g CL, 8.2 ± 1.7 g SL, ANOVA, F = 3.39). Thereafter, at weaning on day 21 (46.9 ± 5.4 g CL, 60.4 ± 10.6 g SL, SNK $p < 0.05$), at day 60 (321 ± 27 g CL, 337 ± 27 g SL) and the experimental day (455 ± 74 g CL, 481 ± 59 g SL, $p < 0.05$), SL rats were significantly overweight.

PrRP significantly activated PaMP neurons of controls (n = 64, paired t-test $p < 0.05$) as shown with an example in *Fig. 1A*, whereas PRL had no significant effect on the mean firing rate (n = 42, $p > 0.05$), although there was a greater proportion of neurons inhibited (firing rate reduced by more than 20% as shown in *Fig. 1B*) than activated. In *Fig. 2* the proportions of responsive neurons are summarized, as well as those activated in contrast of those inhibited by the drugs. Orexin-A (OX-A) had some activating and some inhibiting effects on PaMP neurons of controls. The seemingly activating effect of orexin-B (OX-B) (*Fig. 2*) did not reach a significant level in terms of mean firing rate of all neurons investigated due to a number of neurons reducing the firing rate without reaching the 20% level (change of the mean discharge rate from 2.17 to 2.22 spikes/s, n = 26). No significant differences (ANOVA and Chi^2 $p >$

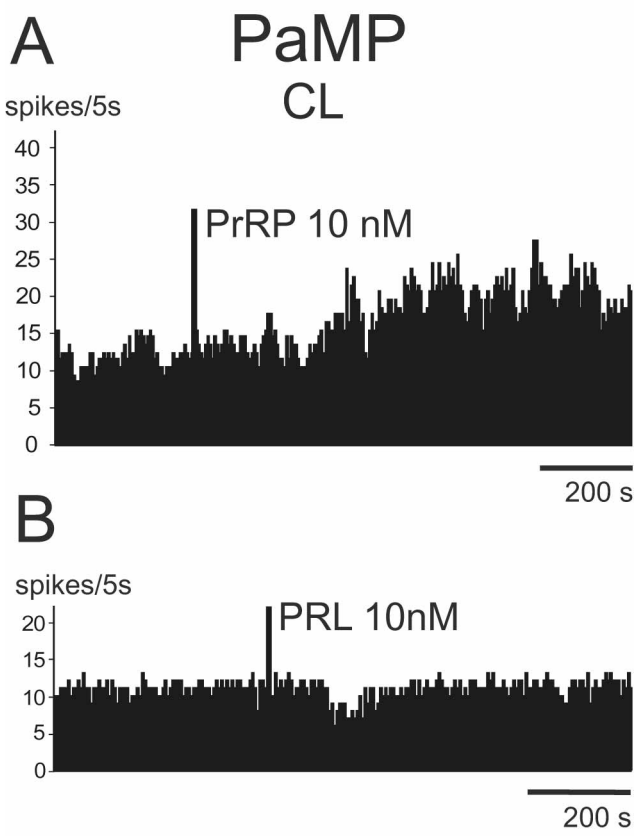

Figure 1 (A and B). Frequency-time histograms of activity of two paraventricular neurons (PaMP) studied in brain slices of control rats (CL). A: Prolactin-releasing peptide (PrRP) induced a prolonged activation. B: Prolactin (PRL) induced an inhibition. Abscissa: time, ordinate: spikes/ 5 s. The administration of a drug is marked by a vertical bar (concentration is that in the drops).

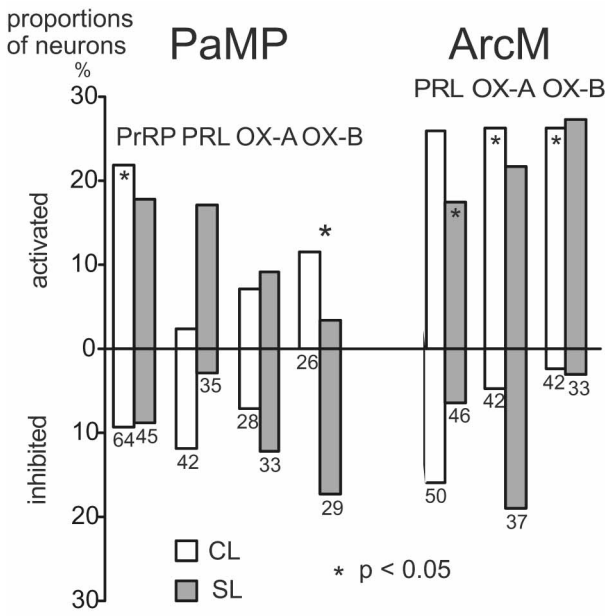

Figure 2. Proportion of neurons activated and of neurons inhibited by prolactin-releasing peptide (PrRP), prolactin (PRL), orexin-A (OX-A) and orexin-B (OX-B), respectively, in the medial parvicellular part of the hypothalamic paraventricular nucleus (PaMP) or in the medial part of the arcuate nucleus (ArcM). The asterisk within a column marks the significance of the difference of drug-induced firing to background firing (paired t-test), the asterisk besides two columns shows the significant difference between the neuronal groups (controls, CL; small-litter rats, SL) in the effect of the drug (ANOVA, SNK). The number of neurons studied is given below the columns.

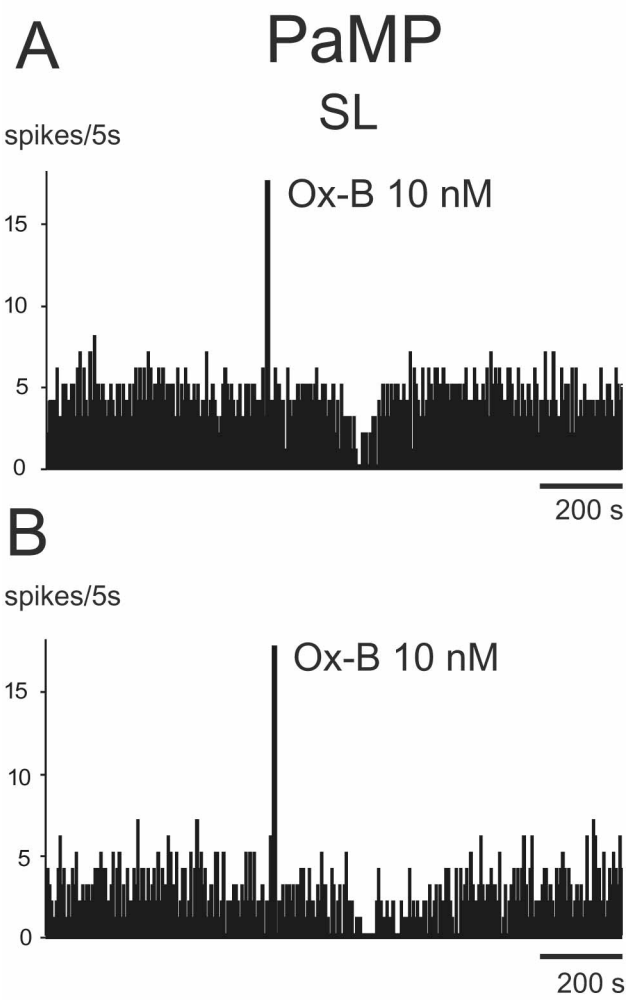

Figure 3 (A and B). Firing rates of two PaMP neurons of SL rats. A and B: Orexin-B suppressed firing. Denotations as in Fig. 1.

0.05) occurred in PaMP neurons of SL rats as compared to controls with regard to their responses to PrRP, OX-A and prolactin, although again the latter showed a trend towards more activating effects in terms of the proportion of neurons affected (*Fig. 2*). However, PRL changed the mean firing rate of SL neurons only from 2.03 to 2.06 spikes/s (n = 35). In contrast, there was a significantly different effect of OX-B on PaMP neurons: the main effect changed from activation in controls to inhibition in SL rats (ANOVA, SNK $p < 0.05$). The suppressive action of OX-B on two SL neurons is shown in *Fig. 3A* and *3B*. The mean firing rate changed from 1.86 spikes/s (baseline) to 1.67 spikes/s (n = 29).

OX-A and OX-B significantly activated medial arcuate neurons of controls, while PRL did so in overweight SL rats (*Fig. 2*). CL and SL rats did not differ in the responses of ArcM neurons to these peptides. Examples of the effects are shown in *Fig. 4*.

Discussion

The effects of the peptides observed in normal weight control rats correspond to results of other authors. Prolactin has been found to activate neurons in the arcuate [16] and to increase there c-Fos, but not in proopiomelanocortin (POMC) neurons [3]. Orexins are described to activate NPY neurons in the arcuate [32,38,51]. NPY neurons are predominantly located in the medial part of the arcuate [14] where we recorded from. Therefore, we would expect that orexigenic peptides activate NPY-synthesizing ArcM neurons. These neurons can also synthesize GABA and inhibit POMC neurons which in turn are located mainly in the lateral arcuate [20,21,22]. An activation of GABAergic neurons in the arcuate by orexins has been reported [2], but orexins can also directly reduce calcium within POMC neurons via OX_2 receptors [32]. These effects can further contribute to an increase in food intake (for review, see [22]). Direct administration of orexins into the arcuate increased food intake [32]. The effects of these peptides did not differ from those observed in overweight SL rats in our study, although ArcM neurons of a great part of these rats were differentially affected by the anorectic CRF_2 (corticotropin-releasing factor) receptor agonist stresscopin-related peptide [8].

Orexigenic substances could be expected to inhibit paraventricular neurons, because lesion of the PVN or inhibition by GABA is reported to induce food intake [25,37]. In our study, PRL inhibited a great part of responsive neurons in controls, but the inhibitory effect did not reach the level of statistical significance in terms of mean neuronal activity. In the supraoptic nucleus, both inhibitory and excitatory effects were observed [50]. In female rats, administration of PRL into the PVN is described to induce food intake [42]. In male rats, PRL seems not to affect feeding [18], but to be involved in stress responses [13,27]. On the other hand, orexins are described to increase c-Fos in the PVN [4], although in electrophysiological recordings iontophoretically applied orexin-A had no significant effect on neurons that were activated by the anorectic leptin [44]. We observed in a small proportion of neurons both activation and inhibition. Orexin-B in the micromolar range activated nearly 80% of PVN neurons (45). Neurons of the PaMP express mainly the OX_2 receptor [29] which has affinity to both OX-A and OX-B. Administration of orexins into the PVN did not induce food intake [49]. The activation of PVN neurons [45] and increase of c-Fos [4] coincide with reports of increased energy expenditure after administration of orexins [28,53]. As mentioned, missing of orexin is followed by hypophagia, but also obesity [15]. Neurons of the PaMP include a population synthesizing CRF. The change in the effect of orexin-B to reduced activation and increased inhibition could lead to reduced activity, e.g., of CRF producing neurons, thereby reducing energy expenditure in overweight SL rats. A possible mechanism for this change in action of OX-B could be a change in the location of receptors in connection with GABAergic neurons and terminals as well as synaptic alterations in terms of plasticity [20]. OX-A and OX-B are known to have differential effects on metabolism and thermogenesis and to use except different receptors also different mechanisms [53]. This could be a possible cause of the different effect on PVN neurons in our study. It is to mention that we did not observe

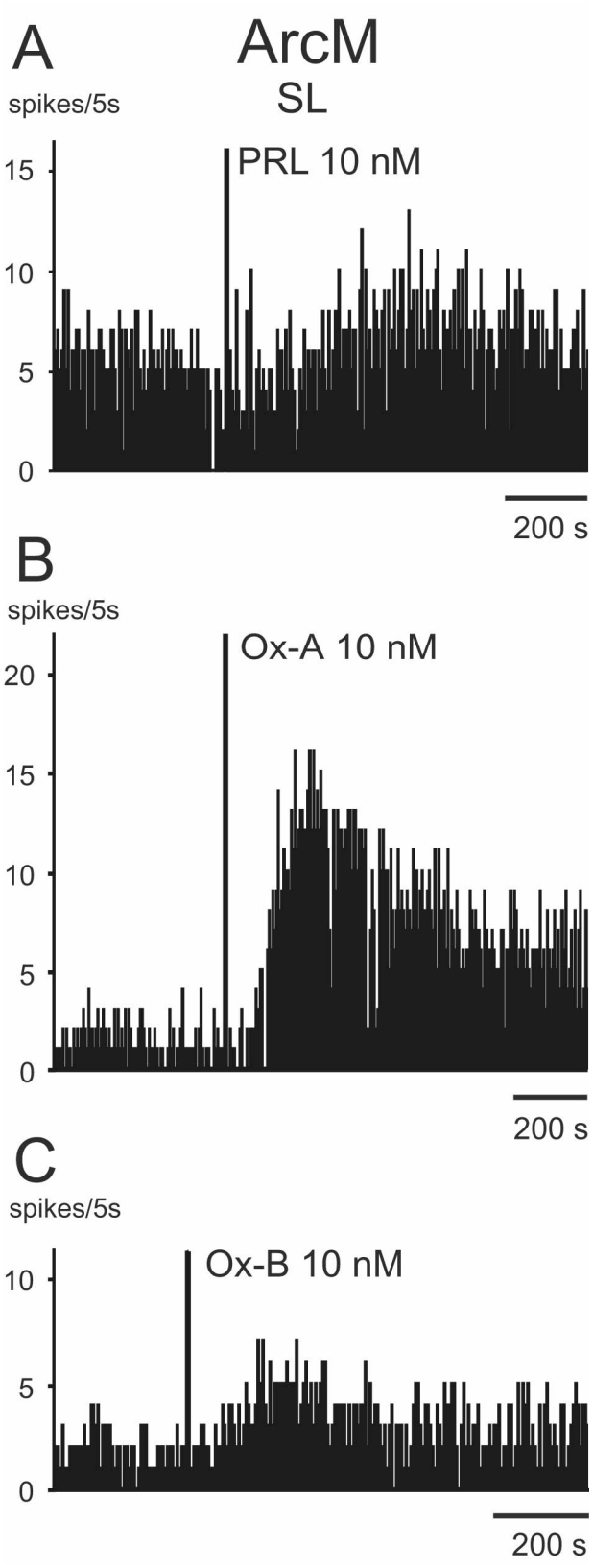

Figure 4 (A, B and C). Firing rates of three ArcM neurons studied in brain slices of SL rats.
The peptides prolactin, orexin-A and orexin-B activated the neurons. Denotations as in Fig. 1.

a change in the action of orexin-A on ventromedial hypothalamic neurons in overweight SL rats compared to controls [17].

PrRP was described to induce c-Fos protein in the paraventricular nucleus [31] and to increase the release of CRF [31]. There are some discrepancies between data reported in the literature. The anorectic response to PrRP could not be evoked by administration into the PVN and seems to be mediated by the DMH [43], but corticotropin antagonists blocked the food intake reducing effect of the peptide [24]. In rats, PrRP (1–31) has been shown to bind in the nanomolar range to its specific receptor [19,40]. While a single administration of PrRP reduces food intake, repeated administrations do not [11], although there are also contradictory results [52]. Generally, the peptide is thought to act only in the short term range, which is a possible explanation for the missing differences between control and overweight SL rats in our study.

Taken together, adult rats that are permanently predisposed to overweight due to early postnatal overfeeding did not show significant changes in responses of the studied hypothalamic neurons to prolactin, PrRP and orexin-A, but their hypothalamic paraventricular neurons responded with increased inhibition to orexin-B. This increase in inhibition by orexin-B, however, could in vivo contribute to reduced energy expenditure of overweight SL rats. These results indicate that changes acquired during early development of neuronal responses to feeding relevant peptides are not a general non-specific mechanism of neurochemical plasticity, but concern specific hypothalamic nuclei and/or neuropeptides.

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