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THE EFFECT OF FOOD INTAKE ON THE CENTRAL MONOAMINERGIC SYSTEM IN THE SNAIL, *LYMNAEA STAGNALIS**

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We investigated the effect of food intake on the serotonin and dopamine levels of the CNS as well as on the spontaneous firing activity of the CGC in isolated preparations from starved, feeding and satiated animals. Furthermore we investigated the effects of 1 μM serotonin and/or dopamine and their mixture on the firing activity of the CGC. The HPLC assay of serotonin and dopamine showed that during food intake both the serotonin and dopamine levels of the CNS increased whereas in satiated animals their levels were not significantly more than the control levels. Recording from the CGC in isolated CNS preparation from starved, feeding or satiated animals showed that feeding increased the firing frequency of the CGC compared to the starved control. The application of 1 μM dopamine decreased the firing frequency whereas the application of 1 μM serotonin increased the firing frequency of the CGC. We conclude that during food intake the external and internal food stimuli increase the activity of the central monoaminergic system and also increase the levels of monoamines in the CNS. Furthermore, we also suggest that the increased dopamine and serotonin levels both affect the activity of the serotonergic neurons during the different phases of feeding.

Keywords: Dopamine – serotonin – CGC – feeding – *Lymnaea*

INTRODUCTION

Behaviorally, initiation of feeding needs a critical level of the feeding arousal as well as activity in feeding central pattern generator network (CPG). A basic question of feeding is how the CPG responsible for the generation of rhythmic feeding movements controlled by command neurons or modulators [4, 19, 26, 48]. Cyclic activity of the buccal feeding network (fictive feeding) can be triggered by the increased activity of interneurons extrinsic or intrinsic to the central pattern generator (for rev. see Elliott and Susswein [4]). In *Lymnaea* the 5HT-containing cerebral giant cell (CGC) and its homologs in other gastropod species are capable of initiating fictive feeding in isolated CNS as in *Lymnaea* [22, 24] *Pleurobranchea* [6] and *Helisoma*

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[7]. Although *in vivo* recording from freely behaving animals showed that CGC in *Lymnaea* [10, 51] and its homolog the MCC *in Aplysia* [5, 20, 47], increased their activity during the generation of feeding movements. However, extirpation experiments [30] suggested that these cells did not drive the feeding system, but only modulated the feeding motor program (for rev. see also [4, 19, 26]). Dopaminergic neurons have also been reported to play a role in the feeding CPG. Buccal dopaminergic neurons were also able to initiate and maintain feeding cycles in *Aplysia* [11, 43] and *Helisoma* [29]. Additionally, in *Aplysia* the increased activity of a cerebro-buccal dopaminergic interneuron was also able to initiate a feeding cycle of the buccal CPG [31]. These observations indicate that the feeding CPG can be commanded by dopamine-containing neurons. Extracellularly applied DA and 5HT also exerted marked effects on the feeding CPG in isolated CNS of various gastropods. The DA-induced rhythmic feeding motor programs in reduced preparations was similar to that evoked by feeding stimulants in *Limax* [50], *Helisoma*, [29, 44], *Lymnaea* [16, 45] and *Aplysia* [12, 43]. Externally applied 5HT on isolated CNS preparations excited the buccal motor neurons and in cases was able to induce rhythmic buccal motor output in *Limax* [50], *Helisoma* [7], *Aplysia* [12] and *Lymnaea* [16, 24, 45].

In *Lymnaea* only one serotonergic neuron, namely the CGC but no dopaminergic neuron have been identified as exerting modulatory effects on the activity of feeding CPG. However, as feeding is a complex behavior which may require the participation of the whole body of the animal, it is more likely that the whole monoaminergic system takes part in the feeding behavior rather than just the CGC and a few dopaminergic neurons. There are numerous DA-immunostained [3] and 5HT-immunostained [14] neurons in the CNS of *Lymnaea*.

To see the activity of the monoaminergic system during feeding, we measured the 5HT and DA levels in the circumesophageal ganglion ring (CNS) from starved, feeding and satiated animals, respectively. We also recorded the spontaneous activity of the CGC in isolated CNS-buccal ganglia preparations made from starved, feeding and satiated animals. Finally we tested the effect of the extracellularly applied 5HT and DA and their mixture on the activity pattern of the CGC neuron.

MATERIALS AND METHODS

Adult specimens of the snail *Lymnaea stagnalis* were used for the experiments at spring. The experimental animals, were divided into three groups: (i) starved animals which did not receive food for three-four days, (ii) animals which were feeding until the experiment, (iii) satiated animals.

HPLC assay of monoamines

Monoamines (5HT, DA) were measured from the CNS of starved, feeding and satiated animals (n = 5).

The monoamines were assayed by a Waters high performance liquid chromatograph equipped with an electrochemical detector. After removing the shell the CNS were dissected and homogenized in 0.1 M perchloric acid and centrifuged.

Aliquots of the clear supernatant were injected into reverse phase C18 Nucleosil column. The mobile phase contained 0.05 M sodium acetate, 0.2 M EDTA, 1 mM octanesulfonic acid, 10% methanol and the final pH was adjusted to 4.0 with citric aid. The flow rate was 1.0 ml/min and the column temperature was 24 °C.

Intracellular recording from the CGC

The isolated CNS including the paired buccal ganglia were dissected from starved, feeding and satiated animals, respectively, and the outer layer of the connective tissue sheath was removed mechanically from the cerebral ganglia. The inner layer was softened using protease solution (SIGMA XIV). Thereafter the preparations were kept rest for 1 h before using for the experiments.

The visual identification of the CGC on the ventral surface of the cerebral ganglia was based on their location, size and coloration [23]. The CGCs were impaled with a standard glass microelectrode, and their intracellular activity recorded by standard microelectrophysiological method (for detailed description see [49]).

During the experiments the isolated CNS was continuously perfused with standard *Lymnaea* saline [49], and freshly made solutions of both 5HT and DA were applied into the bath through the same perfusion system.

RESULTS

HPLC assay of monoamines

In the CNS of starved animals the HPLC assay showed the presence both DA (38.98 ± 1.59 pmol/mg) and 5HT (26.7 ± 2.51 pmol/mg) (Fig. 1A). The food intake (30–40 min) induced more than 50% increase of both DA (65.57 ± 3.95 pmol/mg vs. 38.98 ± 1.59 vspmol/mg) and 5HT (54.85 ± 3.43 pmol/mg vs. 26.7 ± 2.51 pmol/mg) levels (Fig. 1A). However, when satiated (10–30 min after termination of feeding) the levels of both DA and 5HT decreased, with their values close to the control. (DA: 34.95 ± 3.05 pmol/mg; 5HT: 31.84 ± 2.43 pmol/mg) (Fig. 1A).

The values of the DA/5HT ratios (Fig. 1B), which numerically express that DA or 5HT is dominant at a given time, during the different arousal states show that in starved animals there is a marked DA dominance (1.47 ± 0.15). During food intake although both DA and 5HT content markedly increased in the CNS, the DA dominance significantly decreased (1.2 ± 0.12) and reached its minimum by satiation (1.1 ± 0.1) indicating that by satiation DA and 5HT were present nearly in the same concentrations.

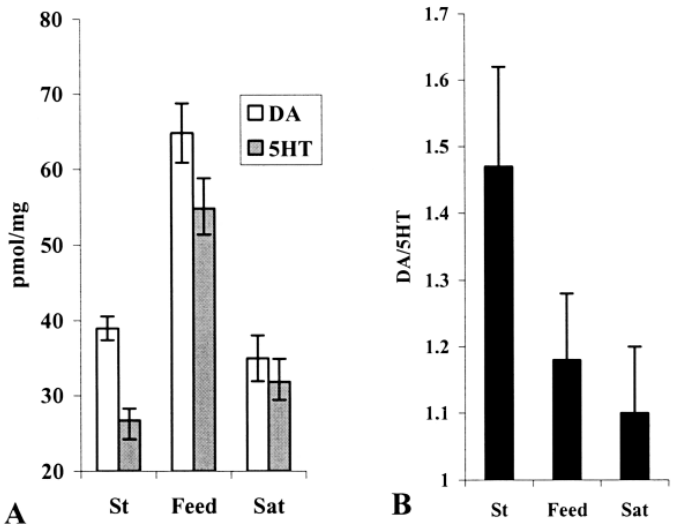

Fig. 1. A. HPLC measurements of DA and 5HT contents in the CNS of starved (St), feeding (Feed), and satiated (Sat) animals (mean ± SEM values). B. The value of DA/5HT ratio in the CNS from starved (St), feeding (Feed) and satiated (Sat) animals (mean ± SEM values)

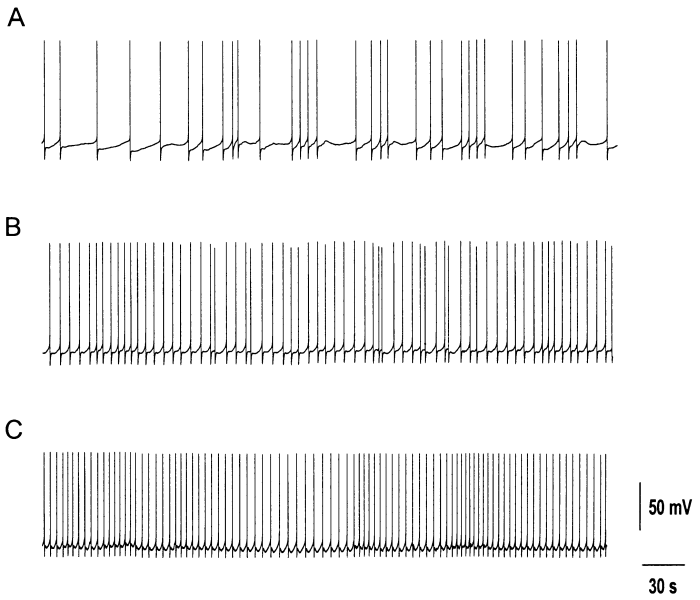

Fig. 2. Intracellular activity of the right and left CGCs in reduced CNS-buccal ganglia preparations made from of starved (A), feeding (B), and satiated (C) animals. Activity is recorded from 4 to 8 min after electrode penetration

The activity pattern of the CGC in starved, feeding and satiated animals

In the isolated CNS-buccal ganglia preparations made from starved animals the CGC showed variable firing pattern, either tonic but occasionally phasic (bursting) firing activity (Fig. 2A). The CGC from feeding animals always showed tonic (regular beating) firing pattern, moreover, the firing frequency was higher than in starved animals (Fig. 2B). The CGC in preparations from satiated animals showed tonic firing activity (Fig. 2C) and its frequency was also higher than in starved animals.

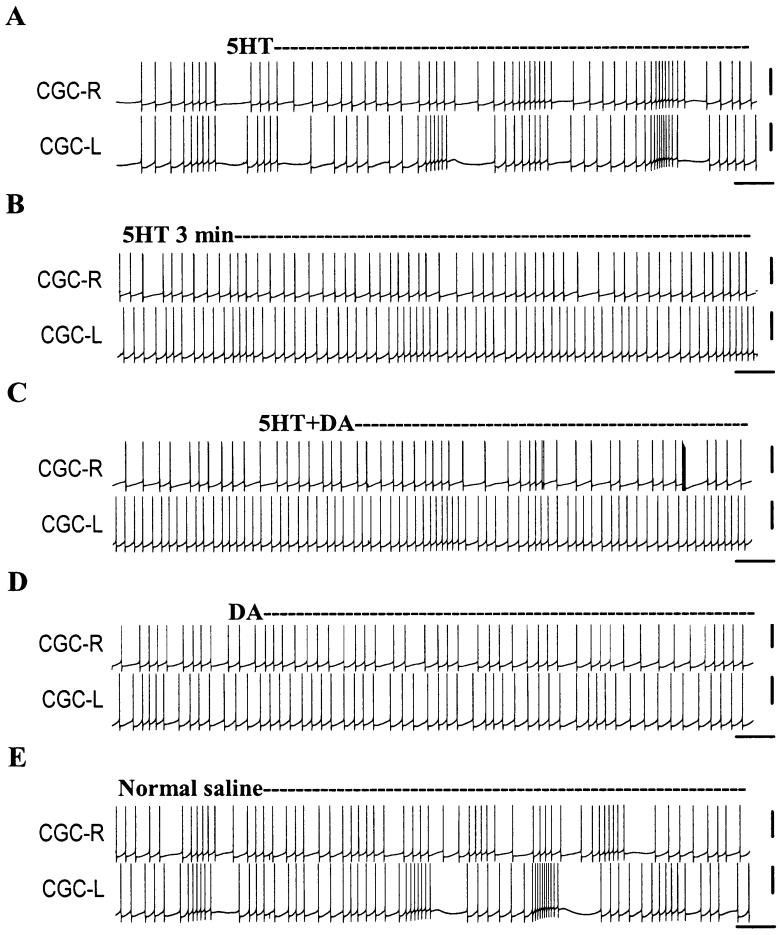

Fig. 3. The effects of 5HT, 5HT and DA, as well as DA on the activity pattern of the CGC in isolated CNS-buccal ganglia preparations made from starved animal. Both 5HT and DA were used in 1 μM concentrations

The effects of DA and 5HT application on the activity pattern of the CGC

5HT (1 μM) applied over isolated CNS-buccal ganglia preparations made from starved animals, changed the firing pattern of the CGC neurons which became tonic and fired more rapidly (Fig. 3A, B). When 5HT and DA was applied together (both in 1 μM concentration) following the 5HT application, the activity pattern of the CGC did not change (Fig. 3C). However, if thereafter only DA (1 μM) was added without washing out, the firing frequency of the CGC markedly decreased (Fig. 3D). The 5HT and DA effects could be washed out (Fig. 3E).

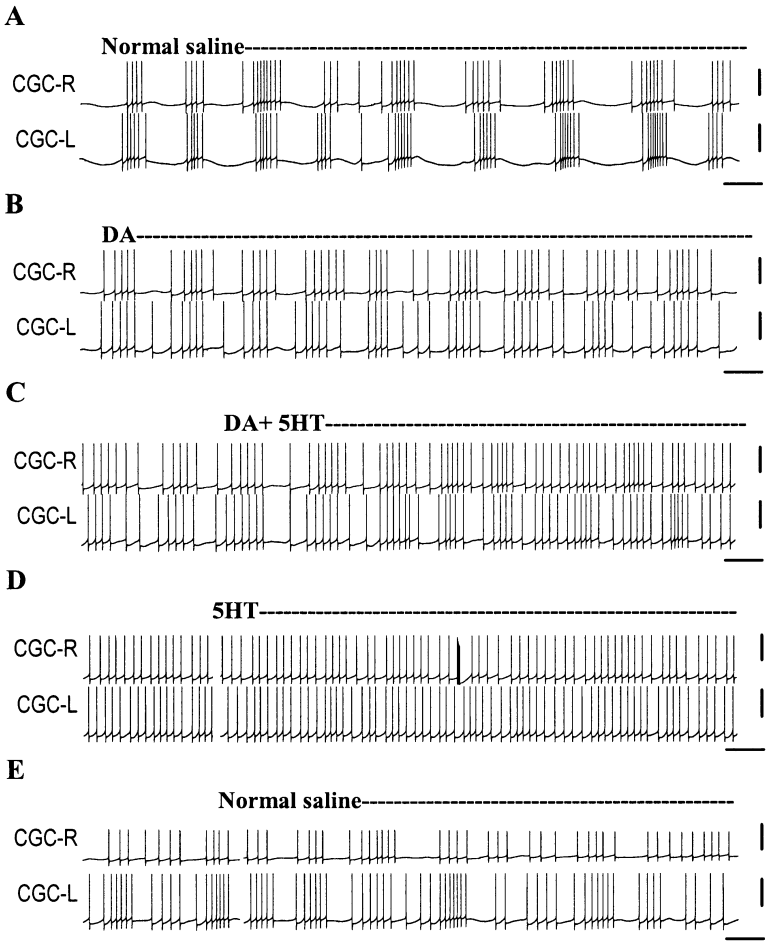

Fig. 4. The effect of DA, DA and 5HT, as well as 5HT on the activity pattern of the CGC in isolated CNS-ganglia preparations made from starved animal. Both 5HT and DA were used in 1 μM concentrations

When DA was applied first (1 μM) its effect on the spontaneous firing activity of the CGC was more variable: the action potential generation of CGC either slightly increased, or more often decreased (Fig. 4A, B). If thereafter 5HT and DA were applied together in the same (1 μM) concentration the firing frequency of the CGC always increased (Fig. 4C). When the bath was perfused with 1 μM 5HT without washing out, the firing frequency slightly further increased (Fig. 4D). The DA and 5HT effects could be washed out (Fig. 4E).

DISCUSSION

Our HPLC assay of monoamines shows that during food intake both 5HT and DA levels increased in the CNS but decreased nearly to the control level by satiation. Additionally, the activity of the CGC in reduced CNS-buccal ganglia preparations from feeding animals were higher than from the control starved animals. These observations suggest that during the food intake the activity of both the serotonergic and dopaminergic systems increased. At the level of behavior, the external food stimuli elicited feeding arousal in both *Aplysia* [18, 37, 48] and *Lymnaea* [45] which lead to food intake (for rev. see also [4]). Additionally the bulk of the swallowed food particles in the gut provided an internal food stimulus, which at sub-satiated state, also increased the feeding arousal [19, 38].

In semi-intact preparations, feeding stimulants applied to the oral area increased the activity of the extrinsic feeding modulator serotonergic cerebral neurons (CGC in *Lymnaea* and the MCC in *Aplysia)* and in parallel, increased the activity of the feeding CPG [13, 15, 32, 35, 43] for rev. see also [4]. *In vivo* recordings from *Aplysia*, and *Lymnaea* showed that the activity of the MCC or the CGC correlated well with feeding arousal. They increased their firing activity markedly during the protraction phase of the feeding cycle [5, 20, 47, 51]. Feeding stimulants also excited the buccal DA containing neurons which neurons were intrinsic to the feeding CPG in *Aplysia:* [11, 32, 43] and *Helisoma* [29] and these interneurons were able to initiate and maintain the buccal feeding program. These observations of increased activity of both 5HT and DA containing neurons during food intake may contribute to the elevated 5HT and DA levels in the CNS during feeding. The internal food stimuli (distension of the gastrointestinal tract) close to satiation decreased the feeding arousal and contributed to the termination of feeding in *Aplysia* [9, 21, 37, 39]. These inhibitory stimuli may be responsible for the decreased activity of the monoaminergic system leading to the decrease of both 5HT and DA levels of the CNS at satiation.

Our observation in isolated CNS preparations that the CGC fires faster in feeding than in starved animals, correlates well with the *in vivo* observations that during food intake the CGC increases its firing activity [51]. The faster CGC firing during feeding, coupled with increases in firing due to externally applied 5HT, suggest that *in vivo,* a positive feedback loop will occur during feeding. This kind of positive feedback has been described from isolated 5HT neurons of *Lymnaea*. If the 5HT content of a 5HT neuron was elevated by the injection of 5HT or its precursor tryptophan,

the spiking activity of the neuron markedly increased [1, 2]. Our present observation in isolated CNS preparation that the firing activity of the CGC was markedly higher in feeding than in starving animals confirms this suggestion of positive feedback. DA (1 μM) applied to the bath most often decreased the firing frequency of CGC, whereas 5HT always increased the firing frequency even in the presence of 1 μM DA. When 1 μM DA was applied following the application of the same concentration of 5HT, the firing frequency of the CGC decreased. The DA application alone may correspond to the DA dominance assayed in the CNS of starved animals which resulted in a low firing frequency of the CGC in isolated preparations. The application of the mixture of 1 μM DA and 5HT or the 5HT alone may correspond to the situation assayed during food intake and satiation when the DA dominance continuously decreased. The recordings from the CGC during these circumstances show that the presence of 5HT increases the firing frequency of the CGC. These observations suggest that the levels of liberated DA and 5HT during the different phases of feeding (see the results of the present HPLC assay) can mutually determine the firing activity of not only the CGC but the whole population of 5HT neurons. Although we have not tested the effects of 5HT and DA on identified DA neurons we may not exclude that their firing activity can also be modulated by 5HT and DA mutually during the different phases of feeding.

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