THE MODULATORY EFFECT OF SOME NEUROACTIVE SUBSTANCES ON THE RELEASE OF ADIPOKINETIC HORMONE FROM THE CORPUS CARDIACUM OF *LOCUSTA MIGRATORIA* 

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**Summary**

The release of the adipokinetic hormones I and II from the neurosecretory cells within the the glandular lobes of the corpus cardiacum is under the synaptic control of axons running through the paired nervus corporis cardiaci II. The neurotransmitter(s) involved is/are as yet unknown. cAMP is known to be involved as a second messenger. The effect of the putative neurotransmitters octopamine, dopamine, tyramine and serotonin on the adipokinetic hormone release was tested in an *in vitro* incubation system. These neuroactive substances had no effect on this release on their own, but they potentiated the hormone release induced by cAMP enhancing agents, like IBMX and forskolin.

**INTRODUCTION**

Adipokinetic cells situated in the glandular lobes of the corpus cardiacum (CCG) of the African migratory locust, *Locusta migratoria*, synthesize three adipokinetic hormones (AKH's)(Oudejans et al. 1991). AKH I and II are colocalized in the same secretory granules (Diederen et al. 1987). They are released during flight and are involved in the mobilization of lipids from the fat body (Beenackers et al. 1984). During prolonged flight, lipids are the main fuel for the flight muscles. The secretory pathway and function of AKH III are still unknown (Oudejans et al. 1991). The secretion of AKH I and II is initiated by a group of neurons in the lateral part of the protocerebrum (Rademakers 1977). Their axons run through the paired nervi corporis cardiaci II (NCC II), making direct synaptic contact to the adipokinetic cells in the ipsilateral glandular part of the corpus cardiacum (CC). Neurosecretory cells situated in the pars intercerebralis of the protocerebrum have their axons running through the NCC I. These axons branch in the neurohemal part of the CC (CCS).

Electrical stimulation of the NCC II *in vitro* results in the release of AKH I and II. Although electrical stimulation of the NCC I on its own has no effect on the release of
AKH, it potentiates the effect of NCC II stimulation (Orchard and Loughton 1981). Moreover, severance of both the NCC I and II is required to prevent lipid mobilization during flight (Bloemen 1985). These results imply that NCC I activity does not initiate AKH release, but rather plays a neuromodulatory role.

Orchard et al. (1993) provided evidence for octopamine (OA) to be the neurotransmitter initiating AKH release from the glandular cells with cAMP as a second messenger. Konings et al. (1988a) however, using an antiserum against OA, could not detect any immunoreactivity in the lateral cells of the protocerebrum, the NCC II and the CC. Consequently, up till now it is uncertain whether OA is the neurotransmitter or not. Two other neurotransmitters were immunocytochemically demonstrated to be present in the pars intercerebralis, the NCC I and the CCS: serotonin (5HT) (Konings et al. 1988b) and dopamine (DA) (Vieillemande et al. 1984). Tyrasine (TA) was reported to be present in the lateral cells of the protocerebrum, the NCC II and the CCG of the American cockroach, Periplaneta americana (Eckert, personal communication). Although TA is a precursor for OA, Downer et al. (1993) provided evidence that it is a neurotransmitter on its own. Nevertheless, TA has not yet been demonstrated in the corpus cardiacum of the African migratory locust.

The present study reports on the effects of the above mentioned neurotransmitter substances in the absence or presence of a cAMP activating agent on the release of AKH in vitro.

MATERIAL AND METHODS

Animals

For all experiments adult male specimens of the African migratory locust, Locusta migratoria, were used 14 days after their final moult. The animals were reared under controlled conditions of temperature (30 °C), relative humidity (40 %), a photoperiod of 16 hours light, and on a diet of reed grass supplemented with rolled oats.

Chemicals

Octopamine (OA), serotonin (5HT), dopamine (DA), tyramine (TA), 3-isobutyl-1-methylxanthine (IBMX) and forskolin (FK) were purchased from Sigma, St Louis, USA. All components of insect saline buffer (ISB) were from Merck GmBH. Acetonitrile (HPLC-grade) was purchased from Rathburn chemicals ltd.

In vitro incubations

CC were excised and collected in pools of 5 in 200 µl ISB (150 mM NaCl, 10 mM KCl, 4 mM CaCl₂, 2 mM MgCl₂, 10 mM HEPES, pH 7.0). After rinsing three times with ISB, the pools were incubated in 200 µl ISB under continuous and moderate shaking in a moist chamber for 30 min. at 35 °C. The incubation media were collected in Eppendorf tubes and diluted 1:1 with 2.5 M HAc. The CC were rinsed once and then incubated under the conditions as described above in 200 µl ISB provided with test agent(s) with or without IBMX or FK. IBMX and FK enhance the intracellular level of
cAMP. To test the viability of the CC, occasionally, a high concentration of potassium (100 mM) was applied during the second or even during a third incubation period. Also after the second period the incubation media were collected in Eppendorf tubes and diluted with 2.5 M HAc (1:1). The amount of AKH I released into the incubation media, was measured directly with reversed phase high performance liquid chromatography (RP-HPLC) (C18 sierenorb 250 x 4 mm, 5 μm). Fluorescence was detected with a spectrophotometric detector Shimadzu RF-10A, ext. 276 nm; em. 340 nm). The column was eluted with a rising gradient of acetonitrile. The ratio between the amount of AKH I released during the second period (induced release) and during the first period (spontaneous release) was calculated.

The watchglasses (on which the incubations took place) and the pipette tips were siliconized with 1% di-methyl-chlorosilane diluted in chloroform.

RESULTS

According to Figure 2, the spontaneous release of AKH I during the second incubation period was slightly higher than during the first period (1:1.4). There was a large increase of the AKH I release after depolarization of the plasmamembrane by a high potassium concentration (100 mM).

IBMX stimulated the AKH I release in a dose-dependent manner. As can be seen from Figure 1, it achieved its maximum effect already at a concentration of 10 μM, having its halfmaximum effect at a 5 μM concentration. The effect of FK on the AKH release was also dose-dependent (Fig. 1). The maximum effect was achieved at a concentration of 25 μM FK. The ratio between the test and the control incubation increased from 1.4 to 5.2.

![Figure 1](image_url). Dose response curves of IBMX and FK respectively. Increasing concentrations of IBMX and FK were present during the second incubation period.
All four neuroactive substances had no effect on their own. In the absence of a cAMP activator, no elevation of the AKH I release took place, compared to the control experiments. In the presence of IBMX however, OA increases the IBMX-induced AKH I release slightly, whereas 5HT has a strong enhancing effect. It can be seen from Figure 2 that DA an TA had equal effects on the AKH I release somewhere in between the levels of OA and 5HT.

Figure 2. Effects of OA, DA, TA and 5HT alone and in the presence of 500 μM IBMX. Con: the second incubation medium consisted of ISB only. K: 100 mM potassium was added to ISB during the second incubation period. n is number of experiments ± SEM.

DISCUSSION

Isolated CC always spontaneously release AKH I into the incubation medium. The absolute amount released during the first 30 minutes is about 2 pmol per CC; during the second period the amount is approximately 3 pmol per CC (Fig. 2). This is higher than the release in vivo, which is estimated at about 0.1 pmol. It may be the consequence of denervation of the CC. It is more probable however, that this spontaneous release in vitro is caused by the fact that the bathing medium (ISB) contains no trehalose. It is well described that trehalose, which is abundantly present in locust hemolymph, has an inhibiting effect on the AKH I release (Bloemen 1985). The finding that high potassium concentrations induce a strong release of AKH I (Fig. 2), demonstrates that during the experiments, the adipokinetic cells react properly to a stimulating stimulus.

Orchard and Loughton (1981) demonstrated that electrical stimulation of the NCC II axons in vitro results in an elevation of cAMP levels in the AKH cells in the corpus cardiacum. The results of the experiments with IBMX as well as with FK support this hypothesis. AKH I release is stimulated in a dose-dependent manner after adding the cAMP enhancing agents (Fig. 1). The maximum effect of FK is somewhat higher than the effect of IBMX, but the two drugs intervene on a different enzyme involved in the cAMP pathway. Pannabecker and Orchard (1986) described a subthreshold concentration for IBMX of 50 μM. Figure 1 shows that, in our in vitro system, the maximum effect on the release of AKH I is achieved at a 5 times lower concentration, with a subthreshold as low as 5 μM. It seems, that our detection system is much more sensitive than the bioassay used by Orchard et al. (1983a) and Pannabecker and Orchard (1986). The dose
response curve of FK is comparable to the one obtained from experiments with vertebrate brain (Bender and Neer 1983). Our results support the hypothesis of Orchard et al. (1983b) that cAMP is involved in the process of AKH I release.

From the data obtained from pharmacological experiments, Orchard et al. (1983a) concluded that the neurotransmitter released from the NCC II axon terminals in the CCG is OA. As shown in Figure 1, OA on its own has no effect on the AKH I release in vitro, but it enhances the AKH releasing effect of IBMX. Orchard et al. (1983a) performed all pharmacological experiments with OA and other agents in the presence of 500 µM IBMX. This suggests that Orchard et al. (1983a) described an OA receptor, which is not the receptor used by the natural occurring neurotransmitter in the NCC II axon terminals. Moreover, Konings et al. (1988a) could not detect any anti OA-immunoreactivity in the CC nor in the NCC I and NCC II nor in the dorsolateral part of the protocerebrum where the somata are located of the secretomotor neurons that innervate the AKH cells in the CCG. Taking these results together, the supposition that OA is the neurotransmitter, used by the NCC II axons is questionable. Rather, since OA is present in the hemolymph as a stress hormone (Orchard et al. 1981), it may act as a humoral factor, having a positive modulatory action on an already elevated AKH I release.

Konings et al. (1988b) provided immunocytochemical evidence for the presence of 5HT in the pars intercerebralis, the NCC I and the CCS; no immunoreactivity was found in the NCC II or in the CCG. The same holds true for DA (Vieillemaringe et al. 1984). 5HT as well as DA are not able to induce AKH I release on their own, but, the IBMX induced release is enhanced by DA and more strongly by 5HT. This is consistent with the findings that electrical stimulation of the NCC I alone has no effect; it enhances the effect of electrical stimulation of the NCC II. 5HT and DA may, therefore, serve as neurotransmitters present in the NCC I axons modulating the process of AKH I release.

The effect on the AKH I release of TA, which is described to be present in the CCS and CCG of Periplaneta americana equals the effect of DA. Up till now, there is no evidence for TA being present in the CC of Locusta migratoria.

REFERENCES


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