POSSIBLE INVOLVEMENT OF MONOAMINES
IN THE RELEASE OF ADIPOKINETIC HORMONE IN THE
LOCUST SCHISTOCERCA GREGARIA

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SUMMARY

1. The adipokinetic hormone release, which can be induced by anti-
cholinesterases, is reduced by depleting the content of monoamines in the
nervous system.

2. The participation of monoamines in the pathway of release of adipo-
kinetic hormone is studied in vivo and in vitro.

3. A possible mechanism for anticholinesterase-induced release of this
hormone involving cholinergic and aminergic transmission is postulated.

INTRODUCTION

Aminergic neurones (Dahlstrom, 1973) are present in the nervous system of insects
and the monoamines contained in them act as neurotransmitters (Pitman, 1971). The
brain of Schistocerca gregaria contains intraneuronal dopamine, noradrenaline and
5-HT (Klemm & Axelsson, 1973) and the corpus cardiacum of the same insect has
varicose fibres containing dopamine and 5-HT (Klemm, 1971).

The glandular lobes of the corpus cardiacum of Schistocerca contains the adipoki-
netic hormone, which on release elevated the level of lipid in the haemolymph (Golds-
worthy, Mordue & Guthkelch, 1972). Insecticide poisoning brings about the abnormal
release of this hormone at the paralytic stage (Samaranayaka, 1974). The participation
of acetylcholine in organophosphate-induced release of adipokinetic hormone has been
demonstrated (Samaranayaka, 1975b). Catecholamines participate in the release of
gonadotropins in mammals (Schneider & McCann, 1970) and it is possible therefore,
that monoamines are involved in the release of adipokinetic hormone from the corpus
cardiacum.

The above possibility has been studied pharmacologically, (a) by using drugs which
are known to deplete monoamines from their storage sites, (b) by testing the effects of
drugs which block dopamine and 5-HT receptors, and (c) by looking at the effects of
monoamines and their agonists on the release of adipokinetic hormone. Although the
interpretation of the results of such pharmacological experiments presents some
difficulties, it has, nevertheless, been possible to suggest a mechanism for anticholin-
esterase-induced release of adipokinetic hormone.

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MATERIALS AND METHODS

Adult male Schistocerca gregaria were taken from laboratory cultures maintained at 35 °C and 30-35% R.H. under crowded conditions with a constant photoperiod and fed on wheat and bran.

In vivo studies

(a) Reserpinization – for depletion monoamines from their storage sites (Gyermek, 1966). Reserpine (Sigma) was dissolved in a drop of ethanol and diluted with physiological saline (Maddrell and Klunsuwan, 1973). Reserpine treated Schistocerca were of two groups. The first received large doses of the drug (250 μg g⁻¹ 10 μl⁻¹), over a relatively short period of time, the injections being repeated four times at regular intervals for 18–20 h. The other group received small doses of the drug (50 μg g⁻¹ 10 μl⁻¹) four times at regular intervals for 48 hours. In both instances insects were maintained under laboratory conditions and no food was available.

(b) Monoamine agonists and antagonists. These were dissolved in physiological saline. Two injections were made, 1·5–2·0 and 0·5 h prior to treatment with insecticide chemicals.

In (a) and (b) Schistocerca was treated topically (Samaranayaka, 1974) with the following compounds:

(1) Organophosphate - Baythion (an emulsifiable concentrate containing 500 g/l phoxim) from Bayer Agrochemicals;
(2) Carbamate-Zectran;
(3) Pyrethroid-NRDC 119 (cismethrin) from Rothamstead Experimental Station.

Haemolymph lipid was estimated just before poisoning and at prostration according to the method of Goldsworthy et al. (1972). The drugs tested were the α receptor antagonist, phentolamine mesylate (CIBA); the β receptor antagonist, DL-propranolol HCl (Sigma), the monoamine antagonists, cyproheptadine HCl (Sigma), the monoamine antagonists, cyproheptadine HCl (Sigma), the monoamine antagonists, cyproheptadine HCl (Sigma), the monoamine antagonist, apomorphine HCl. The monoamine antagonists and the agonist were gifts from Dr A. S. Horn, MRC Neuropharmacology Unit, Cambridge.

In vitro studies

These were done on the isolated corpus cardiacum (Samaranayaka, 1975b). 3-Hydroxy tyramine HCl (Sigma) and 5-HT, creatine sulfate complex (Sigma) were used to stimulate the corpus cardiacum in vitro. They were tested in the presence and absence of the monoamine oxidase inhibitor, pargyline HCl (Abbot Laboratories).

RESULTS

(a) Reserpinization. Individuals which had been pretreated with reserpine for 18–20 h and were then poisoned with baythion or zectran failed to show a significant increase in haemolymph lipid compared to the controls (Fig. 1). Reserpine treatment,
Table 1. The effectiveness of corpus cardiacum (C.C.) extracts in releasing lipid

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) C.C. injected into insects treated with 500 μg g⁻¹ reserpine</td>
<td>+130·27 ± 30·6</td>
</tr>
<tr>
<td>(b) C.C. from reserpinized insects injected into untreated locusts</td>
<td>+186·36 ± 20·99</td>
</tr>
<tr>
<td>(c) Control: C.C. injected into untreated locusts</td>
<td>+190·11 ± 33·5</td>
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</tbody>
</table>

The figures given are for the percentage change in haemolymph lipid concentration and expressed as the mean ± s.e. Number of observations indicated in parentheses.

however, did not prevent the release of adipokinetic hormone with the pyrethroid, NRDC 119 while prolonged reserpinization prevented baythion-induced release of adipokinetic hormone (Fig. 1).

Catecholamines increase lipolysis in mammalian adipose tissue, an effect which involves the participation of adrenergic receptors (Sawin, 1969). If a similar system operates in insects (i.e. catecholamines act as hormones), then it is conceivable that reserpine could block these receptor sites non-specifically so as to prevent the release of lipid into the haemolymph. This possibility was eliminated by treating locusts with a large dose of reserpine and then injecting extracts of corpora cardiaca (from untreated locusts) which had been incubated in high potassium saline (Samaranayaka, 1975b) (Table 1). In another experiment, locusts were treated with 1·5 mg reserpine for 24 h. Their corpora cardiaca were removed and incubated in 80 mM [K⁺] saline; 50 μl of this medium was assayed for adipokinetic hormone (Table 1). The results show that reserpine neither blocks receptors on the fatbody nor does it deplete the corpus cardia-cum of its content of hormone.

Since the depletion of monoamines with reserpine was without effect on the pyre-throid-induced lipid release (Fig. 1) only organophosphates and carbamates were used in subsequent experiments. Dopamine, noradrenaline and 5-HT all failed to initiate an adipokinetic response. Similar results were obtained with dopamine agonist, apomorphine. Baythion poisoning of apomorphine (10⁻⁴ M)-treated locusts caused an increase in concentration of blood lipid of 120·15 ± 5·04%, which suggests that the agonist did not potentiate lipid release.

In contrast to the lack of effect of agonists, antagonists of monoamines were very effective in reducing anticholinesterase-induced lipid release (Figs. 2–4). The effects of α and β adrenergic blockers are shown in Fig. 2. Phentolamine and propranolol were able to reduce the release of lipid induced by zectran to a level that was statistically significant when compared with control insects. Dopamine and 5-HT receptor blockers were more potent in limiting hormone release. Chlorpromazine and α flupenthixol produced a very significant reduction (P < 0·001) in lipid release with baythion and α flupenthixol a similar effect (P < 0·02) with zectran. In a rather similar fashion, 5-HT antagonists, methysergide and gramine produced significant reductions (P < 0·001 and P < 0·01 respectively) in the elevation of blood lipid in baythion and zectran treated insects. Treatment with cyproheptadine, however, did not inhibit the increase in haemolymph lipid on poisoning with either baythion or zectran when compared to untreated controls. To see if these antagonists affected the lipid releasing ability of the fatbody an experiment similar to that done with reserpine was repeated on individuals which had been pretreated with methysergide (10⁻³ M and 10⁻⁴ M). The
Fig. 1. Effect of reserpine on lipid release. Percentage increase in lipid release (mean ± S.E.) in response to (left to right), (BO) baythion only; (RL + B) large dose of reserpine followed by baythion; (RS + B) small dose of reserpine followed by baythion; (ZO) zectran only; (RL + Z) large dose of reserpine followed by zectran; (PO) NRDC 119 only; (RL + P) large dose of reserpine followed by NRDC 119; (RO) reserpine only. Dose of insecticide was 500 μg g⁻¹ 5 μl⁻¹.

Significance in this and all subsequent experiments was determined by Students’ t-test. In each case comparison has been made in the increase in haemolymph lipid between drug treated and non-treated control insects treated with the same insecticide. The number of observations is given in parentheses.

Concentration of blood lipid rose (an average of 186.3 and 127.1% respectively) in these insects showing that there was no impairment at the level of the fatbody.

The corpus cardiacum could not be stimulated in vitro with dopamine or 5-HT either in the presence or absence of pargyline. In an attempt to facilitate the penetration of drug molecules into the corpus cardiacum, the osmotic pressure of the saline was increased to shrink the cells and thus enlarge the intercellular spaces, but this had no effect. However, entry of drugs is probably not rate limiting, because acetylcholine can stimulate the isolated glands to release adipokinetic hormone (Samaranayaka, 1975b). Since acetylcholine stimulated the glands in vitro an attempt was made to see if this acetylcholine-induced release could be blocked in vitro by dopamine antagonists but these experiments gave inconsistent results.
Adipokinetic hormone release in \textit{S. gregaria} 419

![Graph](image)

\textbf{Fig. 2.} Effect of \(\alpha\) and \(\beta\) receptor antagonists on lipid release. Percentage increase in lipid release (mean \pm s.e.) in response to (L to R), (BO) baythion only; (Ph + B) \(10^{-4}\)M phentolamine followed by baythion; (Pr + B) \(10^{-4}\)M propranolol followed by baythion; (ZO) zectran only; (Ph + Z) \(10^{-4}\)M phentolamine followed by zectran; (Pr + Z) \(10^{-4}\)M propranolol followed by zectran.

\textbf{DISCUSSION}

\textit{The locust corpus cardiacum and its innervation}

The corpus cardiacum of \textit{Schistocerca} contains dopamine and 5-HT fibres together with neurosecretory fibres (Klemm, 1971). Subsequently, Klemm & Axelsson (1973) identified dopamine and 5-HT containing cell bodies in the pars-intercerebralis region of the brain. It is possible, therefore, that these fibres which terminate in the corpus cardiacum have their origins in the cell bodies in the protocerebrum and reach the corpus cardiacum via the nerves NCC 1 and NCC 2. Monoaminergic innervations have been demonstrated in the salivary glands of \textit{Schistocerca} (Klemm, 1972), \textit{Periplaneta} (Bland, House, Ginsborg & Laszlo, 1973) and \textit{Manduca sexta} (Robertson, 1974) Ultrastructurally, this innervation to the salivary glands contains axons with
electron dense granules (Robertson, 1974), resembling 'B' fibres (Knowles, 1967). This possibly explains the relationship between 'B' fibres and monoamine fibres in the corpus cardiacum of Schistocerca (Samaranayaka, 1975a; Normann & Samaranayaka in preparation).

**The effect of reserpine**

Reserpine depletes catecholamines and 5-HT from their storage sites in the nervous system of vertebrates (Dahlstrom & Fuxe, 1965; Gessa, Biggio, Napoleone & Tagliamonte, 1974), insects (Frontali, 1968) and ticks (Megaw & Robertson, 1974). This
Adipokinetic hormone release in S. gregaria

Fig. 4. Effect of 5-HT antagonists on lipid release. Percentage increase in lipid release (mean ± s.e.) in response to (L to R). (BO) baythion only; (Me+B) 10⁻³ M methysergide followed by baythion; (G+B) 10⁻³ M gramine followed by baythion; (Me+Z) 10⁻³ M methysergide followed by zectran; (ZO) zectran only.

Depletion initially occurs in the fibres and terminals and subsequently extends to the cells (Frontali, 1968). In Schistocerca because the corpus cardiacum has catecholamine containing fibres, depletion of amines may well commence here. Pharmacological agents such as insecticides induce the release of adipokinetic hormone (Samaranayaka, 1974); the depletion of monoamines by reserpine inhibited the release of this hormone when locusts were treated with anticholinesterases, baythion and zectran (Fig. 1). In other words, monoamines were necessary for inducing the release of adipokinetic hormone by compounds which act at cholinergic synapses. Pyrethroids are thought to affect axonal transmission (Elliott, 1971) and probably do not interact with acetylcholine receptors. It follows from Fig. 1, that monoamines are not involved in neurohormone release by pyrethroids. It seems that pyrethroids induce hormone release by some indirect means which is not well understood at this stage.

The possibility exists that large doses of reserpine could produce toxic effects that do not result from the depleting action of this drug (Zamis, 1961), which might account...
for the observed inhibition of hormone release. However, even a small dose of reserpine, given over a prolonged period reduced baythion-induced release of adipokinetic hormone (Fig. 1), again indicating the participation of monoamines in the release of hormone.

The nature of the neurotransmitter causing release of adipokinetic hormone

Since reserpine depletes catecholamines and 5-HT from their storage sites, it is not possible to distinguish between catecholaminergic and/or 5-Hydroxytryptaminergic transmitters in this pathway of hormone release. Furthermore, the corpus cardiacum contains both dopamine and 5-HT (Klemm, 1971). In an attempt to distinguish between dopamine and 5-HT receptors, selective agonists and antagonists of these two amines were tested to see in what ways they affected baythion and zectran-induced release of adipokinetic hormone.

The role of catecholamines and 5-HT as transmitters in insects is well established (Berridge & Patel, 1968; House, Ginsborg & Silinsky, 1973). On the basis of the activity of catecholamines on different tissues, two types of receptors, the $\alpha$ and $\beta$ have been proposed (Ahlquist, 1948); there are drugs which specifically block $\alpha$ responses (e.g. phentolamine) and those which affect $\beta$ responses (e.g. propranolol).

Dopamine is thought only to stimulate $\alpha$ receptors (Van Rossum, 1965); if these receptors participate in hormone release, then it follows that phentolamine should suppress this release by virtue of its blocking action (see Fig. 2). As a class of drugs $\alpha$ blockers are not potent dopamine antagonists (see Woodruff, 1971). This may well explain the relatively small reduction in hormone release after phentolamine treatment (Fig. 2). $\beta$ blockers are not thought to affect dopamine systems. It is, therefore, difficult to see why propranolol blocked the baythion-induced hormone release, although non-specific effects cannot be ruled out at this stage.

Effects of monoamine antagonists

Chlorpromazine (Nyback & Sedvall, 1968; Woodruff, 1972; York, 1972; Horn et al. 1974) and $\alpha$ flupenthixol (Møller Nielsen et al. 1973; A. S. Horn, personal communication) act as antagonists of dopamine receptors. Both drugs were effective in blocking anticholinesterase-induced release of adipokinetic hormone (Fig. 3).

Cyproheptadine, methysergide and gramine are antagonists of 5-HT (Gyermek, 1966); injection of $10^{-4}$ M cyproheptadine was without much effect on lipid release, but gramine and methysergide were powerful inhibitors (Fig. 4). Gramine competes with 5-HT receptors in the salivary glands of Calliphora (Berridge, 1972) and methysergide inhibits the response to dopamine as well (Woodruff, 1971).

From the foregoing results the following inferences can be made about the system in Schistocerca.

(a) A dopamine receptor could be the only receptor involved because all the dopamine antagonists blocked hormone release; the effects of the 5-HT blockers could be considered as non-specific and unrelated to the actual mechanism of hormone release;

(b) Both dopamine and 5-HT receptors could participate in the release process. Under these circumstances, antagonists would act on either one or both receptors in varying proportions depending on the potency of the particular drug for example the
Figure 5. A possible mechanism for anticholinesterase-induced release of adipokinetic hormone.

Adipokinetic hormone release in S. gregaria

Atropine, curare

Reserpine

Baythion, zectran

Antagonists

‘Barrier’

DA 5-HT

hormone

ACh

Eserine

High [K+] ^

Dopamine is a transmitter in the brain of *Helix aspersa* (Kerkut, Sedden & Walker, 1966, 1967). Woodruff (1971) has constituted a tentative model of a composite dopamine/5-HT receptor. A composite receptor of this nature is in agreement with the results of this investigation on *Schistocerca*.

In vitro studies

Although the corpus cardiacum is the most likely site for the location of this receptor, it was not possible to stimulate the glands *in vitro* with monoamines. One explanation for this may be that monoaminergic nerve endings are deeply seated in the gland and are well separated, from the circulating haemolymph containing catecholamines (Karlson & Herrlich, 1965), by physical and/or chemical barriers. It is possible that transmitters are broken down by enzymes in the gland, and as a consequence never reach the receptor sites as has been demonstrated with glutamates in *Schistocerca* (Clements & May, 1974). Similarly, dopamine is the transmitter in the salivary glands of *Manduca sexta* but isolated glands could not be stimulated by catecholamines (Robertson, 1974). On the contrary antagonists seem to penetrate this barrier partly because they are not metabolized by enzymes and also because they are more lipid soluble.

Pharmacological agents other than insecticides did not elicit an adipokinetic response *in vivo*. The explanation for this may lie partly in the ability of the Malpighian tubules to excrete ‘foreign’ substances such as atrophine at a rapid rate (Maddrell & Gardiner, 1976) and partly due to the existence of breakdown and detoxification mechanisms in the insect. For instance in *Calliphora* nearly 60% of injected 5-HT is rapidly excreted (M. J. Berridge, personal communication). Of course, there is also considerable dilution of the drug inside the insect, for mature male *Schistocerca* has a blood volume of 390.72 ± 16.93 μl (B. O. C. Gardiner, unpublished observations) (i.e. when 20 μl of a drug at a concentration of 10⁻⁴ M was injected its concentration *in vivo* would be of the order 5.0 x 10⁻⁶ M).

A model for the possible mechanism for anticholinesterase-induced release of adipokinetic hormone is illustrated in Fig. 5. The primary site of action of organophosphates and carbamates is the cholinergic synapse, where acetylcholine receptors have nicotinic and muscarinic properties (Samaranayaka 1975 b). Activation of these receptors leads to the stimulation of aminergic fibres which innervate the corpus cardiacum
(Klemm, 1971). This brings about the release of adipokinetic hormone from the intrinsic cells by exocytosis (Normann & Samaranayaka, in preparation).

In this scheme we see an interesting parallel with the mammalian hypothalamus-anterior pituitary complex. Catecholaminergic and serotonergic nerve endings occur in the hypothalamus (Fuxe & Hokfelt, 1969). The activity of the dopaminergic fibres stimulates the release of follicle stimulating hormone releasing factor and leutinizing hormone releasing factor (Schneider & McCann 1970; McCann et al. 1972). Similarly dopaminergic pathways are involved in the release of prolactin inhibiting factor (McCann et al. 1972). Subsequently MacLeod & Lehmeyer (1974) have provided evidence for the concept that dopamine is the physiological regulator of prolactin secretion and that it acts directly on the pituitary gland. In other words, dopamine itself is the releasing factor; this observation draws a close parallel between insect and mammalian neuro-endocrine systems.

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REFERENCES


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