Neuroendocrine Control of Cleaning Behaviour in *Labroides dimidiatus* (Labridae, Teleostei)

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Abstract

Cleaning symbiosis between *Labroides dimidiatus* and other saltwater fish is not dependent only on visual-tactile stimuli, since the cleaning motivation of the wrasse is strongly influenced by endogenous factors. The application of various neurohormones (neurotransmitters: 5-HT (5-HTP; PCPA), noradrenalin, L-Dopa; tissue hormone: VIP) as well as the opiate-antagonist naloxone, resulted in a change of the actual cleaning time, which possibly indicates the influence of many other interacting hormones of the fish's endocrine system.

1. Introduction

Cleaning symbiosis has been reported for many species, however, without considering the moods (cleaning motivation) of the partners involved. Up until now, more than 40 species of fish have been described to be active in cleaning, where either juvenile, or juvenile and adult animals of one species (obligatory cleaners) inspect strange fish (Brockmann and Hailman, 1976; Matthes, 1978). While interspecific relations among freshwater fish are hardly known (Abel, 1971), several marine teleosts demonstrate a highly ritualized behavioural program with special sign stimuli, and, as far as tropical species are concerned, are found at characteristic points of the reef (cleaning

stations). The ethologically and ecologically best known cleaners are wrasses of the genus *Labroides* (Limbaugh, 1961; Youngbluth, 1968; Losey, 1972; Potts, 1973; Robertson and Choat, 1974). *L. phthirophagus*, which apparently feed only on ectoparasites of other fish, as well as *L. dimidiatus* have been investigated in regard to their function in the tropical reef as contributors to the preservation of health of other species. Other authors believe that this cleaning symbiosis contains commensal and parasitical components, whereas the behaviour in general could be regarded as mutualistic.

The frequently described cleaning action can only then take place, when it is permitted by the mood (motivation) of both the cleaner and the host. Aside from external influences (*L. dimidiatus*: cleaner-livery, dance; host: body-position, decolouration) coordinating the meeting of the animals, endogenous parameters (maturation, hormones) in both participants play an important role. In previous publication I was able to prove that the cleaning motivation of *L. dimidiatus* can be influenced through an altered endocrinal status (Lenke, 1982).

These experiments have demonstrated that the primarily composite behaviour of the hermaphrodite *L. dimidiatus* (fear-hunger; fear-aggression) can be increased in its intensity through either the injection of prolactin itself or its release following the application of metoclopramide. Prolactin (PRL), a hormone originating in the adenohypophysis, has been detected in fish by Walker-Farmer et al. (1977) and Vodicnik et al. (1978). Teleost prolactin has balso been described as paralactin. Its membrane-receptors have been found in several organs (gonads, gills, etc.) (Edery et al., 1984) and in the brain (Blüm and Fiedler, 1974; Hansen and Hansen, 1982). Among other effects, the influence of prolactin on parental care, water and electrolyte balance, lipo- and carbohydrate metabolism as well as its effect on the gonadal activity has been reported (Matty, 1985). Following an application of the PRL-releasers metoclopramide and melatonin an increase of the cleaning intensity was noticed (Lenke, 1982), which could be explained as a result of an intensification of the fish's appetite and a reduction of the fear/aggression-threshold: a calming effect (Spieler et al., 1977; Fiedler et al., 1980). In addition to previous investigations this publication not only discusses the influence of substances that stimulate or inhibit PRL-release with reference to cleaning, but also their effect on ingestion activity.

As in mammals, the PRL-cell activity in fish is regulated through an inhibitory dopaminergic and a stimulatory serotoninergic system (James and Wigham, 1984). In *Anguilla anguilla* injections of serotonin (5-HT

and its precursor 5-hydroxytryptophan (5-HTP) enhanced the PRL-release (Olivereau and Olivereau, 1979); the 5-HT blocking parachlorophenylalanine (PCPA) resulted in reduced PRL-values in the same animal (Olivereau, 1978). The inhibiting effect of dopaminergic neurons in *Gillichthys mirabilis* has been reported by Nagahama et al. (1975) and by McKeown et al. (1980) in *Salmo gairdneri* after applications of L-DOPA, which is known to be the precursor of dopamine. In addition, the catecholamine noradrenaline (NE) has been demonstrated to have a restraining influence on PRL-synthesis, at least in *in vitro* experiments (Clemens and Shaar, 1980). However it is not clear, if NE is able to pass the primitively developed blood-brain-barrier in fish (Knight et al., 1978) and to show biphasic results as reported in mammals (de Wied and de Jong, 1974).

Aside from the neurotransmitters, the opiates play an important role in the regulation of PRL-release. Dupont et al. (1977) state, that β-endorphin as well as met- and leu-enkephalin stimulate the PRL-release, which could lead to an intensified ingestion of food. In order to test this hypothesis I injected the opiate-antagonist naloxone, a drug that is known to have no side effects on other hormones. Application, of naloxone resulted in an inhibition of food intake in rats (Brands et al., 1979) and in *Toxotes jaculatrix* (Piront, 1982), which could possibly be explained as a consequence of an induced PRL-inhibition (Okajima et al., 1980). The prolactin-releasing effects of opioide peptides and various other substances probably originate in a modification of the prolactin-release-inhibiting factor (PIF) and are not due to a direct effect on the hypophysis. Vasoactive intestinal polypeptide (VIP), however, is known to have a direct influence on the prolactin release (Clemens et al., 1980). Because of its close interaction with 5-HT in the hippocampus, VIP is believed to have an important function for learning, memory and other mechanisms underlying stress-responsiveness (Bohus et al., 1982), among various other physiological effects (Rostene, 1984).

The purpose of my current experiments was to further investigate PRL's influence on the cleaning behaviour of *Labroides dimidiatus*.

2. Material and Methods

For these experiments cleaner-fish of the species *L. dimidiatus* (length: 5–7 cm; weight: 2.5–3.5 g) were kept singly and visually isolated in 60 liter tanks at a temperature of $28\pm1°C$. The behaviour of all animals was first investigated during a minimum-3-week observation period. In the course of this acclimation-time, all of the animals' activities occurring during five-minute

periods were recorded in a behavioural protocol three times daily (9:00 a.m.; 1:30 p.m.; 5:00 p.m.). Following the afternoon experiment (1:30 p.m.), the cleaning of a stimulus model was measured by using a stop-watch (presence of the cleaner at the dummy-observation period 5 min). The plastiline-model, which resembled the host fish, was placed approximately 6 cm beneath the water surface in the centre of the tank. Aside from possible accessibility of the dummy from all sides, special care was taken when it came to the posture of the model, which was used during all of the experiments. Only when this experiment was finished, the fish were fed with a mixture of various kinds of frozen food. It should be mentioned, that the value of the untreated animals represents the mean difference of sixty cleaner-fish (20 observations each animal).

After this three week observation period, control-injections of distilled water were applied to each animal (n = 60). The deionisized water was also used as solvent for the active substances. Once a week both drugs and controls were given intramuscularly (μg/gbw) ten minutes prior to the beginning of the observation (at approx. 1:20 p.m.). This period was inserted because in some cases the animals were stressed by the injection-procedure and did not show their natural behaviour. Experimental series were conducted with VIP and naloxone on 4 and 5 animals, respectively. All data were analysed for significance by the student t-test; based on a two-tailed comparison, only data with a p=0.05 were taken into consideration.

Substances used: 5-hydroxytryptamine (5-HT), 5-hydroxy-DL-tryptophan (5-HTP), DL-p-chlorophenylalanine (PCPA), vasoactive-intestinal-polypeptide (VIP) all from Sigma, St. Louis; L-3,4 dihydroxyphenylalanine (L-DOPA) Serva, Heidelberg; L-noradrenalin (Arterenol®; NE) Hoechst AG, Frankfurt; naloxon (Narcanti®) Dupont, Frankfurt.

3. Results

The behaviour of the cleaner-fish *L. dimidiatus* has often been described previously (review: Potts, 1973); here I point out some details that were observed during the dummy-experiments. The so-called cleaner-dance, which introduces the cleaning behaviour, was reported mainly from juvenile animals (Fricke, 1965), which were observed in their natural reef environment. The manner of swimming is characterized by a bobbing forward movement, accompanied by a synchronous (labriform) locomotion of the pectoral fins. This behaviour is demonstrated in front of the dummy by juveniles and adults: however during the acclimation-period these tail-flips slowly van-
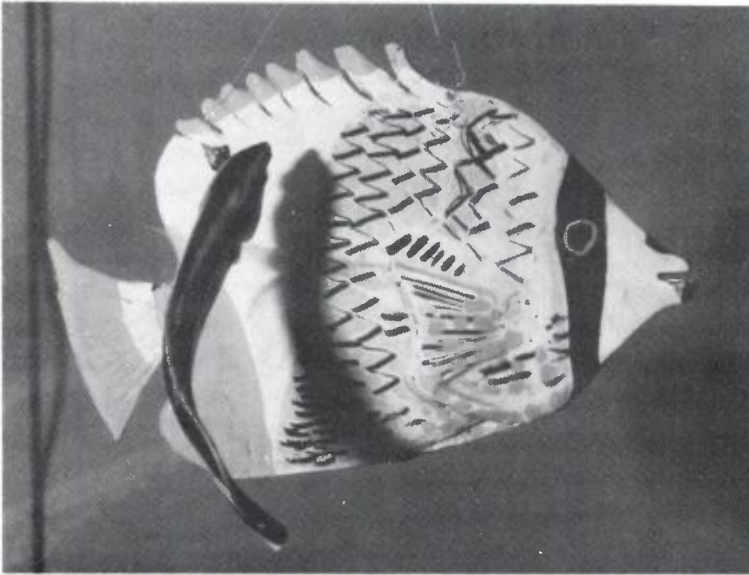
Figure 1. *Labroides dimidiatus* inspecting and searching a dummy for "parasites".

ished between day 5 and 7. After this time, *L. dimidiatus* swam directly to the presumed host (Fig. 1) and began the typical cleaning action without any introduction. The mean duration of cleaning in a given time of 5 min amounts to approx. 46 sec (= 15.5%; n = 60), and it should be mentioned that the wrasse's satiation is of secondary importance (see also Lenke, 1982). Previous experiments with live hosts have shown that the cleaner was very soon aware of the parasite-infection rate of its aquarium companions and therefore was actively cleaning only occasionally. This led to an experimental condition giving the wrasse a rather short time-period at the dummy. The duration of the model-investigation is limited, because there is no reaction of the model to tactile stimuli of the cleaner (live hosts react on tactile stimuli with spreading of the unpair fins). Control-injections (c) applied after the acclimation-period did not significantly change the mean cleaning time (47 sec = 15.9%; n = 60).

Compared to the controls, 4 concentrations of 5-HT (Fig. 2 below) led to a significant decrease of the cleaning activity. However, a high (more than average) cleaning intensity was observed daily following the hormone's injection. The typical cleaner-dance which again appeared after the injection with
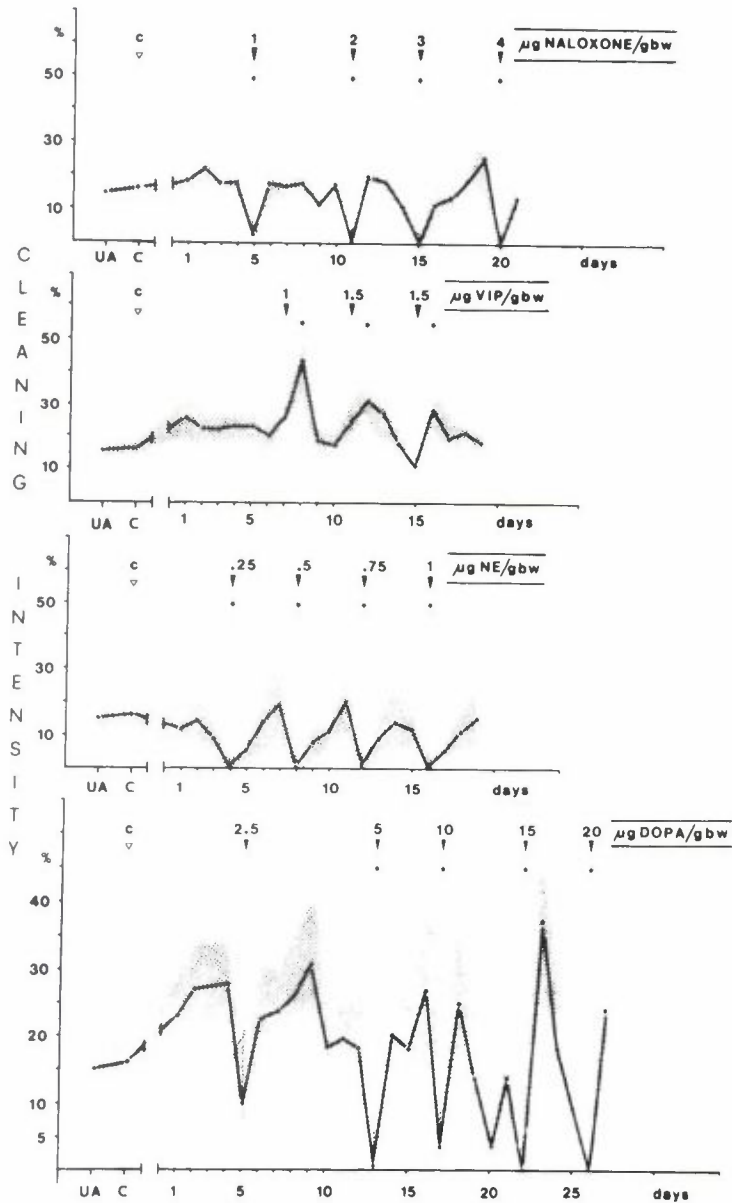
Figure 2. Effect of intramuscular injection of serotonin (5-HT), 5-hydroxytryptophan (5-HTP) and parachlorophenylalanine (PCPA) on cleaning-intensity in *L. dimidiatus*. Abscissa: days of observation. Ordinate: Cleaning intensity in % during 5 min observation time. UA = untreated animals: C = control-injection; statistically significant difference (vs.control) is shown by asterisks (≤ 0.05). The shaded areas represent the standard errors of the mean differences.

increased intensity, implicates an intensive rise in conflict between escape and cleaning. Applications of 5-HTP resulted in increased cleaning compared to the previous day; but there was no significance compared to the controls. Especially in concentrations of 2, 2.5 and 3 μg/gbw, a decline in the investigation of the host was noted during post-observation (Fig. 2 center). Only directly after administration of PCPA, which blocks serotonin-synthesis, a slight elevation of the cleaning activity was noticed (Fig. 2 above). Its antagonistic effects versus 5-HT on the injection day, can not only be recognized in the cleaning but also in swimming activity, where PCPA shows an enhancing influence.

In a range from 5–20 μg/gbw, L-DOPA significantly decreased the cleaning activity, which, however, rapidly increased during post-observations. L-DOPA (Fig. 3), as well as noradrenalin which is synthesized from dopamine, partially suppressed the overall activity significantly on the injection day. Noradrenaline demonstrated its inhibitory effect on cleaning already in low dosages (0.25–1 μg/gbw). In addition, the recovery of the behaviour to normal values, appeared rather slowly (Fig. 3).

Table 1. Effect on cleaning-activity after treatment with several substances. $+$ = Increase; $-$ = Decrease; $*$ = Significant Deviation; 0 = no influence

Substances	effect on cleaning intensity			
	INJECTION DAY		DAY AFTER INJECTION	
5 - HT (SEROTONIN)	−	*	+	
5 - HTP	+		−	*
PCPA	+		O	
L - DOPA	−	*	+	
NE (NOREPINEPHRINE)	−	*	O	
VIP	O		+	*
NALOXONE	−	*	O	

The opiate-antagonist naloxone inhibited the cleaning behaviour of *L. dimidiatus* in all concentrations used (Fig. 3). Additional activities, such as swimming, were negatively influenced only by the highest dosage (4μg/gbw). The cleaning behaviour at the dummy was increased 24 hr after application of VIP, as shown in Fig. 3. It may be concluded, that the
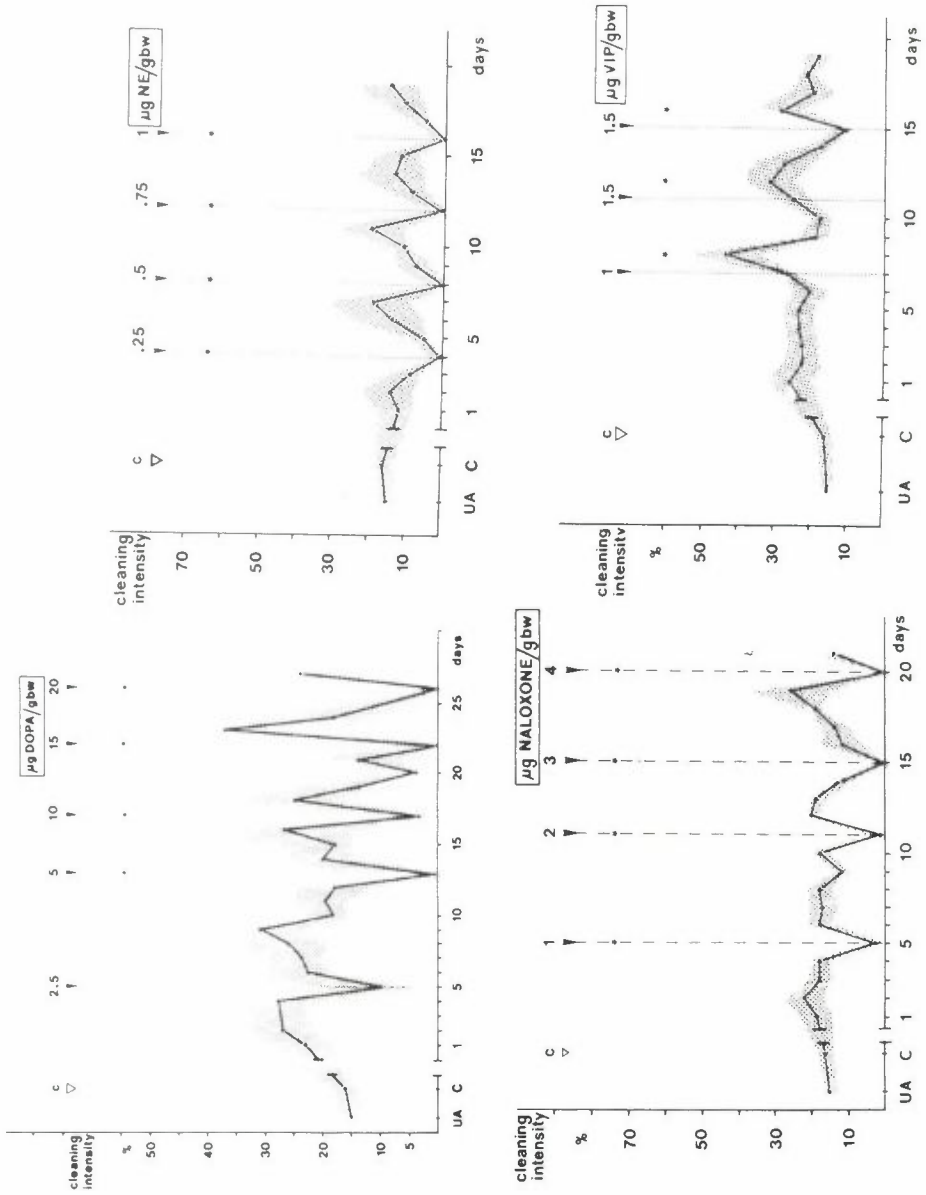
Figure 3. Cleaning-frequency after injection of L-DOPA, noradrenaline (NE), vasoactive-intestinal-peptide (VIP) and naloxone; for explanation see Fig. 2.

cleaner's behaviour faced a reorientation on the injection day. This resulted in occurrence of activities which are normally found in untreated fish that feel completely safe and unobserved (i.e. enhanced inspection of the sleeping caves (Table 1)).

4. Discussion

Injection of 5-HTP or 5-HT increases the PRL-release not only in mammals (Fuller and Clemens, 1981; Jaffe, 1981) but also in fish (*A. anguilla*; Olivereau and Olivereau, 1979). It seems that 5-HT does not easily cross the blood-brain-barrier as does its precursor 5-HTP (in mammals 5-HT cannot pass the blood-brain-barrier). This assumption can be explained through an additional action of the indolamine. Aside from the PRL-cells located in the rostral *pars distalis*, the hypothalamus also controls MSH-cells (MSH = melanophore-stimulating hormone) in the *pars intermedia*. Not only in *A. anguilla* but also in *L. dimidiatus* the injection of 5-HTP induced a more rapid darkening of the skin than 5-HT. Applications of serotonin (0.05, 0.1–0.5 μg/gbw) resulted in a partially significant decrease of the mean cleaning duration, which could be due to a delayed central-nervous 5-HT-PRL-effect followed by a decrease of activity and aggression (Valzelli, 1982). Another explanation could be found in a dopaminergic inhibition induced by the enhanced PRL-level (Perkins and Westfall, 1978). Noted increases on the following day (rebound effect) are most probably caused by the PRL-activity, as this hormone is known to have more than 12 biphasic modes of action (Horrobin, 1980). A rapid PRL-effect can be demonstrated in concentration ranges from 1–1.5 μg/gbw 5-HTP with enhanced duration of the cleaning action on the injection day. This is followed by a continuous decrease resulting in either a complete discharge of the PRL-storage or an (additive, dopaminergic) negative feedback. PCPA inhibits in certain concentrations the tryptophan hydroxylase in the brain of higher vertebrates (Koe and Weissman, 1966) and therefore the serotonin-synthesis which again leads to a reduced PRL-synthesis. In many experiments an influence of PCPA on the duration of cleaning could not be shown. Olivereau (1978) reported less active PRL-cells after injection of PCPA in the eel. It is doubtful that this slightly reduced cell-activity has an effect on the cleaner's behaviour towards its host. In higher vertebrates, Jouvet (1984) was able to prove that PCPA caused a long-lasting sleep deprivation and a shift of circadian rhythms, which had probably influenced the cleaning behaviour in my experiments.

Aminergic and serotoninergic neurons and nerve-fibers have been found in the hypophysis of *Poecilia latipinna* (Groves and Batten, 1985) and *Xiphophorus maculatus* (Margolis-Kazan et al., 1985). In addition, Kitay and Westfall (1976) identified an active uptake of ^{3}H-NE, ^{3}H-DA, ^{3}H-5-HT in the pituitary of various teleosts. ^{3}H-5-HTP and ^{3}H-5-HT were also autoradiographically localized in the pineal organ and the circumventricular areas in *Salmo gairdneri* (Hafeez and Zerihun, 1976). The epiphysis probably interacts with the PRL- and MSH-metabolism through vasotocin (AVT) a hypothalamoneurohypophysic hormone found in fish (Pavel et al., 1975a,b) and indirectly through melatonin, which is regarded to be the AVT-releaser hormone (Pavel and Goldstein, 1979). Melatonin is closely related to serotonin, and proved to have an enhancing effect on the cleaning behaviour (Lenke, 1982).

The PRL-secretion of the hypophysis is controlled in a tonic-inhibitory manner by the catecholamine dopamine. Following the application of its precursor L-DOPA in a concentration of 5–20 μg/gbw, a significant reduced cleaning activity was noticed on the day of injection, which could be caused either by the dopamine itself (Ben-Jonathan, 1980; Flückinger et al., 1982) or by the release of hypothalamic PIF (McCann et al., 1984). In contrary to dopamine, L-DOPA is able to pass the blood-brain-barrier. Behavioural experiments in fish after L-DOPA-injection demonstrated its inhibitory effect on parental fanning in females of *Cichlasoma nigrofasciatum* (50 μg/gbw) and on calling in pairs of *Hemichromis bimaculatus* (25–125 μg/gbw) (Fiedler et al., 1980). It should also be mentioned that L-DOPA seems to have a negative influence on the secretion but not on the actual synthesis (McKeown et al., 1980) in PRL-cells of *S. gairdneri* and various other teleosts (James and Wigham, 1984). This could also explain the high intensity of the cleaning duration on the days following the injection. Noradrenalin, which is synthesized from dopamine, is known to have biphasic activities, which means that the PRL-secretion is promoted by low dosages while high concentrations lead to an inhibition (de Wied and de Jong, 1974). In my experiments, all NE-concentrations used, blocked the cleaning behaviour totally; continuous increases in between injections can be described as a raised melatonin-induced cleaning motivation, as Klein (1978) was able to show the enhanced transformation of serotonin into melatonin in the pineal by noradrenaline.

Aside from neurotransmitters, endogenous peptides with opiatic effects (i.e. β-endorphin) are able to increase the PRL-level without directly influencing the pituitary (Dupont et al., 1977). In addition, these peptides

enhance the overall food intake (Grandison and Guidotti, 1977). The opiate-antagonist naloxone, which I used in my recent investigations, caused an inhi-bition of all cleaning activities in concentration of 1–4 μg/gbw; the frequently described anxiety following naloxone treatment could not be observed. The results presented here reflect the finding of Brands et al., (1979), who could induce an inhibition of food intake in rats after a single naloxone injection. It is also noteworthy, that naloxone prevents a 2-deoxy-D-glucose induced PRL-release according to Okajima et al. (1980). In this respect it is quite interesting that in the same experiments the dopamine-agonist apomorphine has restrained the secretion of PRL.

Together with TRH (Thyreotropin-Releasing Hormone), VIP seems to be the only substance that shows direct effects on the pituitary, where it is re-sponsible for a PRL-release (Frawley and Neill, 1981; Denef et al., 1985). This effect cannot be reversed by dopamine. VIP has been identified among other areas in the brain stem, the hippocampus and the hypothalamus, as well as the intestinal tissue. In my findings the cleaning activity was sig-nificantly increased on the day following the injection of VIP. This effect could result from a stimulated intestinal secretion and perhaps an enhanced glycogenolysis and lipolysis (Sundler et al., 1980).

It can be concluded that, aside from other hormones, PRL is of central importance in the regulation of the cleaning motivation in *Labroides dimidia-tus*. It should be taken into consideration that most effects described above could also be involved in food ingestion.

Acknowledgement

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