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Behavioral Effects of TRH, 3-Me-His^2-TRH and Naloxone in *Hemichromis bimaculatus*

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CHRIST, H. *Behavioral effects of TRH, 3-Me-His^2-TRH and naloxone in* Hemichromis bimaculatus. PHARMACOL BIOCHEM BEHAV 21(5) 727–732, 1984.—Twenty minutes after each injection of TRH (2, 5, 10 μg/g bw), *Hemichromis bimaculatus* displayed frequent chafing (rubbing body on substrate) at rates as high as 65 times in 5 minutes. Chafing continued even after 5 hours. Such frequent displacement activities were not observed in untreated fish. These activities could not be suppressed completely with the opiate-antagonist naloxone. Five minutes after application of 3-Me-His^2-TRH (1, 2, 5 μg/g bw), *H. bimaculatus* was chafing at higher rates compared to those injected with TRH. Calling movements, which are regulated by a very high prolactin level, also occurred in some fish. When naloxone (2, 4, 8, 12, 16 μg/g body weight) was injected, Hemichromis showed excessive spitting and chewing. When 16 μg naloxone was administered, *H. bimaculatus* started to tremble and tried to escape by the presence of a fish net. The fact that chafing was not completely suppressed after naloxone-application implies that naloxone may mediate opiate and non opiate effects [17].

TRH Naloxone Apomorphine Neuropeptides Hormones Prolactin Chafing Displacement activity

TRH is widely distributed in the mammalian brain and has been identified in other vertebrates as well [20].

Evidence of multiple actions of TRH was obtained in humans when it was shown that this peptide was at least equipotent in releasing prolactin and TSH from the pituitary gland and that prolactin release precedes the release of TSH [10]. Moreover, prolactin-sensitive neurons are found in various regions of the brain [1,7].

The hypothalamic releasing and inhibiting hormones were originally described as being responsible for instance for altering the release of anterior pituitary hormones; it is becoming increasingly evident that these peptides also have behavioral actions. For example, hypermobility, tachypnea, trembling of the forepaws, muscle tremor, lacrimation, hyperthermia, piloerection, "wet-dog-shakes" have been observed [9, 11, 12, 16, 24, 25].

The present study was conducted to determine whether TRH would stimulate prolactin release in Hemichromis bimaculatus by inducing behavior patterns caused by high prolactin levels. Since TRH stimulates opiate-like effects as well [15, 24, 25], it was investigated whether certain behavior patterns occurred due to interactions with opiate-receptors.

METHOD

Hemichromis (total length 8 to 12 cm, weight 12 to 16 g) were kept in a rectangular 15 l aquarium of which 3 walls were painted, thereby preventing the fish from seeing each other. After a few days Hemichromis regarded the aquarium as its "territory," which means, he started to fight as soon as another specimen was taken into the aquarium.

In experiments testing fighting behavior, each Hemichromis had to defend its territory against a test fish of about the same size. Size was important since undersized fish (about 1 cm smaller) were frightened, escaped and could not be provoked to fight. On the other hand, oversized test fish (about 1 cm longer) frightened or attacked Hemichromis without the latter fighting back.

At the beginning of an experiment, the fighting intensity of each fish was evaluated until it reached nearly constant levels. This was best achieved when a test fish was presented daily. Observations were recorded once a day, taking 5 minutes for a simple fighting test and another 10 to 20 minutes for recording all the behavior patterns occurring after such a fight. When peptides, naloxone or apomorphine was injected, 5-minute-fighting-tests were conducted several times a day (see "Results"). During such experiments, all charges and thrusts against a rival were counted as one unit. Intention movements, which did not hit the adversary were counted as half a unit [6]. After each fighting test, the behavior of each fish was observed for 10 to 20 minutes; the different behavior patterns were recorded and analyzed.

Before treatment with a peptide or drug, each animal underwent a control injection with distilled water followed by several fighting tests. This was to make sure that the effects were not caused by handling.

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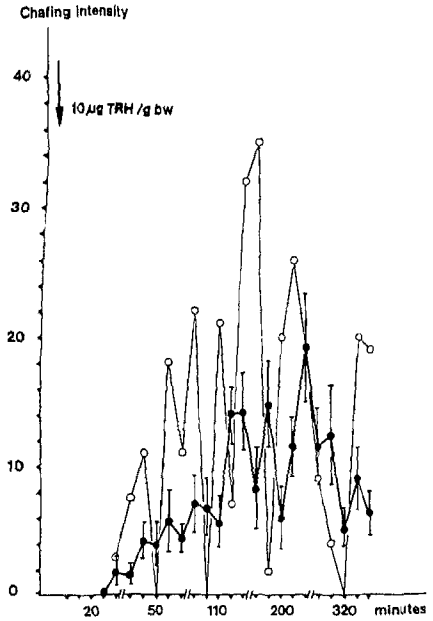

FIG. 1. Chafing intensity of Hemichromis after treatment with TRH. Open circled curve: number of chafing acts for one animal; dotted curve: average values (with standard errors) for 10 animals. Ordinate: "chafing intensity" is standing for the number of chafing acts per 5 minutes. Abscissa: observation time in minutes after the injection. The arrow indicates the time of injection and the dose.

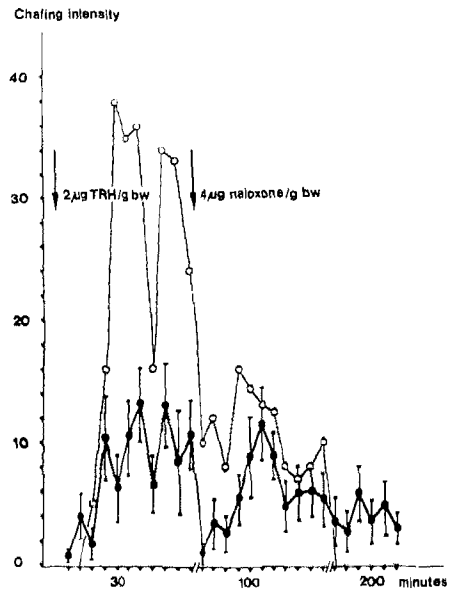

FIG. 2. Chafing intensity of Hemichromis after treatment with TRH and naloxone. Open circled curve: number of chafing acts for one animal; dotted curve: average values (with standard errors) for 10 animals. Ordinate: "chafing intensity" is standing for the number of chafing acts per 5 minutes. Abscissa: observation time in minutes after the injection. The arrows indicate the time of TRH- and naloxone-injection and the dose.

The fish diet consisted of "Tetramin" dry food. The fish were additionally given beef heart at least twice a week. Water temperature was 26±1°C; light per day was 14 hours. All substances were dissolved in distilled water and injected into the dorsal musculature.

The fish were divided into 5 groups:

Group I: 10 Hemichromis were treated with TRH; each fish receiving an injection of 2, 5, 10 µg TRH/g bw at intervals of one week.

Group II: 10 Hemichromis were injected with 2, 5, 5, 10, 25, 50 µg apomorphine/g bw; receiving each dose at intervals of one week. Apomorphine was always given 40–70 minutes after a TRH administration of 2 µg TRH/g bw. A time-span of 40 to 70 minutes was necessary for chafing to occur and for measuring chafing rates.

Group III: 10 Hemichromis were injected with naloxone; each fish receiving 2 and 4 µg naloxone/g bw at intervals of one week. Naloxone was usually given 70 minutes after administration of 2 µg TRH/g bw (see group II).

Group IV: 10 Hemichromis were injected with the TRH-analog 3-Me-His^2-TRH; each fish receiving an injection of 1, 2, 5 µg/g bw at intervals of one week.

Group V: 10 Hemichromis were treated with each 2, 4, 8, 12, 16 µg naloxone/g bw at intervals of one week.

After injection of TRH and 3-Me-His^2-TRH, chafing movements occurred; after injection of apomorphine, circling movements occurred. Since these behavior patterns were not displayed before treatment (Figs. 1, 2 and 4), and because they occurred at very high rates, there was no need for statistical evaluation. However, results between 2 treatment groups (and within the fighting experiments) were analyzed statistically by Student's *t*-test, using two-tailed comparisons.

At the end of the experiment, some fish of each group were sacrificed for histological analyses. The pituitary-prolactin-content was also measured by means of polyacrylamide-gel-electrophoresis (PAGE).

The peptides, drugs and their sources were: TRH, Ferring, Kiel, West Germany; 3-Me-His^2-TRH, Vega, Tucson, AZ; Narcan (naloxone), Endo Laboratories, Garden City, NY; Narcanti (naloxone), Wintrop GmbH, Neu Isenburg, West Germany; apomorphine, Woelm Pharma GmbH and Co, Eschwege, West Germany. (Two different brands of naloxone were used because of availability.)

RESULTS

Group I

About 20 minutes after each TRH-injection, Hemichromis (n=10/dose) displayed frequent chafing (rubbing their bodies on the bottom of the aquarium or on the air stone) at rates as high as 65 times in a 5-minute-observation-session, which still continued after 5 hours (Fig. 1). Such activity was not observed in untreated fish. Injections of 2 and 10 µg TRH/g bw were the most effective in promoting chafing behavior (Figs. 1, 2 and 4). Moreover, differences in chafing intensity were observed when 2 or 10 µg TRH/g bw were injected ($p<0.002$: 10 µg TRH/g bw, Fig. 1, compared with 2 µg TRH/g bw before treatment with naloxone, Fig. 2; $p<0.0001$: 10 µg TRH/g bw, Fig. 1, compared with 2 µg TRH/g bw before treatment with apomorphine, Fig. 4). Inspecting Fig. 2 and Fig. 4, it is interesting to note that 30–60 minutes after injection of 2 µg TRH/g bw a significant difference ($p<0.0001$) in chafing intensity occurred, which will be discussed.

At 2 µg TRH/g bw, chafing was observed as early as 5

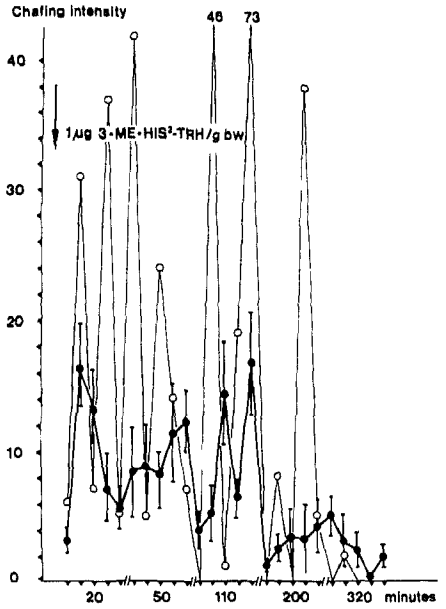

FIG. 3. Chafing intensity of Hemichromis after treatment with 3-Me-His²-TRH. Open circled curve: number of chafing acts for one animal; dotted curve: average values (with standard errors) for 10 animals. Ordinate: "chafing intensity" is standing for the number of chafing acts per 5 minutes. Abscissa: observation time in minutes after the injection. The arrow indicates the time of injection and the dose.

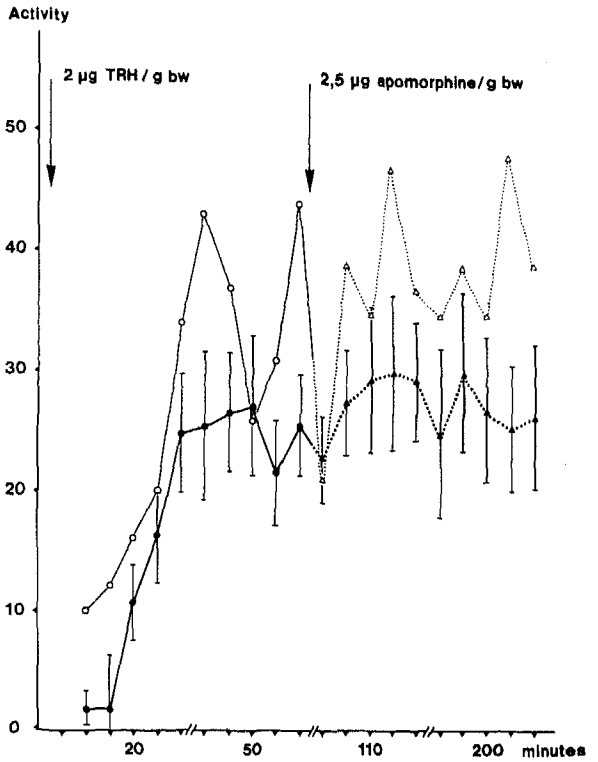

FIG. 4. Chafing intensity of Hemichromis after treatment with TRH and apomorphine. Open circled curve: number of chafing acts of one animal; dotted curve: average values (with standard errors) for 10 animals. Open triangled curve: number of circling acts for one animal; black triangled curve: average values (with standard errors) for 10 animals. Ordinate: "activity" is standing for the number of chafing acts (O—O, ●—●)/circling acts (△ . . . △, ▲ . . . ▲) per 5 minutes. Abscissa: observation time in minutes after the injection. The arrows indicate the time of TRH-apomorphine-injection and the dose.

minutes after injection (Fig. 2 and Fig. 4), whereas when 10 µg/g bw were administered, it took almost 30 minutes for the first chafing to occur (Fig. 1). It is quite interesting to note that 5 µg TRH/g bw elicited hardly any chafing (see "Discussion").

A few minutes before the onset of chafing, Hemichromis would swim excitedly along the aquarium walls. The fish also displayed backward swimming movements followed by a series of chafing movements. Occasionally, "wet-dog-shakes," known as morphine-abstinence-syndrome, were observed. Whenever "wet-dog-shakes" occurred, Hemichromis swam near the surface. The fish would then suddenly move its head with quickly alternating movements, holding its body in a vertical position while pointing its head toward the bottom of the aquarium.

Fighting was impaired for only 30 minutes after each injection (Fig. 5) and hardly any chafing was observed (Fig. 2). Between chafing movements the fish would occasionally jab at the air stone of the aquarium.

Group II

The dopamine-antagonist apomorphine is known to suppress prolactin secretion [18]. In this experiment, it was investigated whether apomorphine, which suppresses prolactin secretion caused by TRH [10, 13, 17], would impede chafing, since chafing might have been caused by an increased plasma prolactin level.

Immediately after the injection of 2.5 µg apomorphine/g bw (see the Method section), chafing was almost completely suppressed ($p<0.0001$; Fig. 4). At the same time, Hemichromis (n=10/dose) displayed circling movements alternating from side to side ([2]; Fig. 4).

Group III

Naloxone reverses opiate-like effects [22], and it is known to reduce prolactin secretions as well [2]. The opiate-antagonist naloxone was injected to investigate whether TRH-induced chafing was caused by a high prolactin level or by an interaction with opiate-receptors. This can be done by recording and analyzing all the different behavior patterns displayed after naloxone-treatment. Since certain patterns are characteristic for morphine abstinence, there are others which are induced by a low prolactin level (see "Discussion"). Naloxone-injections (2 µg/g bw) after treatment with TRH [2] reduced chafing immediately in Hemichromis (n=10/dose). After one week, when TRH was injected again, chafing was displayed at higher rates than the week before. Injections of 4 µg naloxone/g bw (Fig. 2) 70 minutes after TRH-treatment temporarily decreased chafing again ($p>0.01$ for n=1 and n=10). Chafing at higher rates was also observed in group II and will be discussed.

Group IV

Treatment with the synthetic TRH-analog 3-Me-His²-TRH (1, 2, 5 µg/g bw) also induced chafing. In contrast to TRH, chafing occurred as soon as 5 minutes after

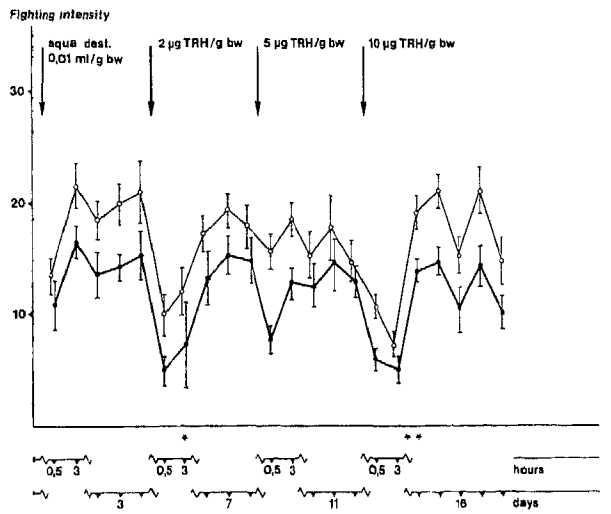

FIG. 5. Fighting intensity of Hemichromis after 3-Me·His²-TRH-treatment. Open circled curve: maximum number of fighting acts (with standard errors) for 10 animals; dotted curve: average number of fighting acts (with standard errors) for 10 animals. Ordinate: "fighting intensity" is standing for the number of fighting acts per minute; abscissa: observation time in hours/days after the injection. The arrows indicate the time of distilled water- (aqua dest.-)/3-Me-His²-TRH-injection and the dose. *=$p<0.025$; **$p<0.05$; * and **refer to the values obtained at the day of peptide-treatment compared with the values obtained at the day of the aqua-dest.-injection. (The maximal and average fighting intensity was obtained by conducting 5-minute-fighting tests, recording each minute separately.)

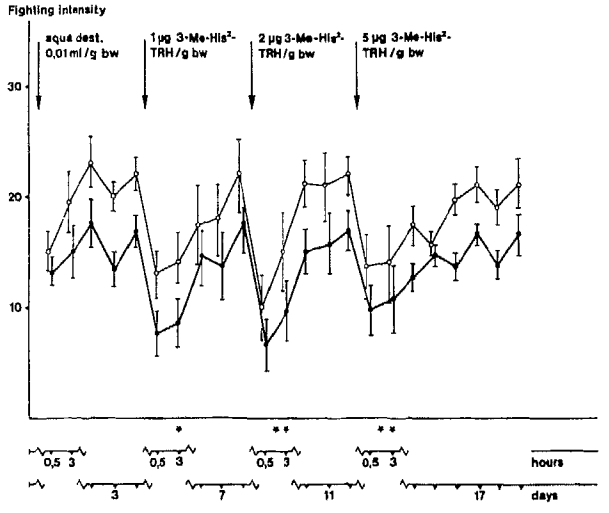

FIG. 6. Fighting intensity of Hemichromis after TRH-treatment. Open circled curve: maximum number of fighting acts (with standard errors) for 10 animals; dotted curve: average number of fighting acts (with standard errors) for 10 animals. Ordinate: "fighting intensity" is standing for the number of fighting acts per minute; abscissa: observation time in hours/days after the injection. The arrows indicate the time of distilled water- (aqua dest.-)/TRH-injection and the dose. *=$p<0.05$; **=$p<0.025$; * and ** refer to the values obtained at the day of TRH-treatment compared with the values obtained at the day of aqua dest.-injection. (The maximal and the average fighting intensity was obtained by conducting 5-minute-fighting tests, recording each minute separately.)

application. Even after 10 minutes, Hemichromis (n=10/dose) chafed at very high rate (Fig. 3), which had not been observed so shortly after injection of TRH. Chafing rates after treatment with 1 µg 3-Me-His²-TRH/g bw were statistically greater ($p<0.0001$ for 1–60 minutes) when compared with an injection of 10 µg TRH/g bw. Hemichromis also displayed chafing up to 73 times in 5 minutes, and high rates of this activity were seen quite often (Fig. 3).

Extremely low chafing rates, as occasionally occurred when TRH was administered, were not observed. When 3-Me-His²-TRH was injected, the fish would not only rub their bodies against the bottom or the air stone, but would chafe themselves in the open water.

Occasionally, 3 fish displayed "calling" behavior in front of air bubbles. This is interesting in that this movement is characteristic of parental care, which is stimulated by prolactin. It may indicate that 3-Me-His²-TRH raises the prolactin level. "Calling" occurred 64 times during a 10 minute time span when 2 µg 3-Me-His²-TRH was injected. After treatment with 5 µg/g bw calling occurred 80 times during the 10 minute observation period.

Occasionally Hemichromis attacked the air stone and displayed "wet-dog-shakes." Fighting activity showed no difference compared to the TRH-experiments (Fig. 6; average fighting intensity: $p<0.05$; maximal fighting intensity: $p<0.02$).

Group V

When naloxone (8, 12, 16 µg/g bw) was injected, fighting activity in Hemichromis (n=10/dose) was impaired for at least three and a half hours. This was not caused by a low readiness to fight, but by inability to attack, which was observed when heavy attacks missed the adversary, the naloxone-treated fish not being able to hit its target. One fish even started to tremble and tried to escape whenever its test fish was presented. This is quite remarkable, since it previously attacked its test fish. Locomotion was reduced. As a consequence, the fish rested on the bottom for several minutes or swam very slowly. Moreover, they displayed excessive spitting and chewing. Occasionally they attacked the air stone of the aquarium, indicating that Hemichromis was still aggressive. Sand digging movements, which had always occurred before the onset of treatment, were reduced. After administration of 16 µg naloxone/g bw, the fish started to tremble and tried to escape when a fish net was presented. When the net was moved back and forth, Hemichromis tried to escape with saltatory swimming movements. Some fish even attempted to make swimming movements while simply resting on the bottom by vigorously moving their pectoral and caudal fins. Despite all these efforts, they remained in place. Similar patterns are known from Crenilabrus as emergency breathing (Fiedler, personal communication); Hemichromis might have been in a similar state. In nearly 70% of the fish, a dark spot appeared where the syringe had penetrated the skin. The spot grew in size until it covered the back completely. This presumably had no effect on the outcome of the experiment. Some fish would suddenly rush through the water, sometimes so vigorously that they would hit the wall of the aquarium head on. Rushing through the water has been reported for fish suffering from ectoparasites

(Fiedler, personal communication). Whether naloxone causes itching which might elicit such reactions has yet to be investigated.

No food intake occurred for 7 hours after each naloxone-injection.

DISCUSSION

As has been shown in the experiments, chafing occurred at high rates after TRH-application. Since TRH increases prolactin secretion [2,10], displacement activity might have been caused by a high plasma prolactin level. However, experiments with fish treated with sheep prolactin revealed that the fish chafed only occasionally [2], suggesting that sheep prolactin is not as effective as teleost prolactin. Moreover, different activities of injected hormones and of hormones released by the endocrine system exist, implying that the released hormones are the more potent ones [13,19]. As has been shown by histological studies [2], TRH is effective in releasing prolactin from the anterior pituitary of Hemichromis. However, it should be considered that chafing may probably be stimulated by other mechanisms as well.

It has been postulated in many publications that the effects of TRH-administration are due to interactions with opiate-receptors [17]. Since naloxone reverses opiate-induced effects, it may be expected that naloxone would suppress opiate-mediated actions. The experiments have shown that naloxone only partially reversed chafing caused by TRH, implying that TRH is not a pure opiate-agonist. Thus, the reduced chafing intensity after treatment with naloxone could be explained by slowly decreasing effects of TRH, which may have been enforced by naloxone. These findings are consistent with experiments which showed that TRH failed to displace ^{3}H-naloxone and ^{3}H-dihydromorphine [15,24]. Since naloxone also reduces the prolactin secretion, a low chafing intensity could be explained by a severe lack of prolactin, indicating that prolactin is one of the several factors needed for this displacement activity. However, the main effect of TRH is mediated by the central nervous system. The sudden onset of chafing and its temporal limitation imply that chafing results from a conflict; therefore chafing should be regarded as a genuine displacement activity [3,5]. Since its normal function is to get rid of ectoparasites, it would hardly take such a long stereotyped course as observed in the experiment.

It has been postulated (see [17]) that high concentrations of TRH produce physiological rather than pharmacological effects, since TRH poorly passes the blood-brain-barrier, which, for instance, is found at the bulbus olfactorius in fish. This might be one reason why injections of 2 and 10 μg TRH/g bw led to similar chafing rates. When 10 μg TRH/g bw was injected, the fish were overtly sedated and calm for the first 30 minutes after the injection. This might be due to the fact that 10 μg TRH passes the blood-brain-barrier and inhibits the firing of TRH-sensitive neurons. This supposition may be regarded as basis upon which displacement activities occur, since in behavioral biology a conflict is induced when two or more behavioral systems are competing each other. Such a competition often leads to quite different behavior patterns, as a result of such a conflict. This does not necessarily imply that displacement activities such as chafing are always dependent on a certain dosage. This has been shown when both 2 and 10 μg TRH/g bw, elicited chafing. It also implies that doses from 2 to 10 μg TRH/g bw might not induce the same behavior pattern, since the behavioral systems activated may be different.

When 5 μg TRH/g bw were injected, the fish were overtly sedated and calm for several hours. This had also been observed for about 30 minutes when 10 μg TRH/g bw was administered, suggesting, to a certain extent, similar reactions. Obviously, 5 μg TRH/g bw was not potent enough to elicit chafing. Since TRH has many binding sites in various regions of the brain [17], it is likely that TRH activates different brain regions simultaneously, perhaps inactivating each other and stimulating a new region.

Apomorphine is known to inhibit prolactin secretion [18]. Although chafing was suppressed after injection with apomorphine, it should not be regarded as a drug-regulating prolactin-mediated displacement activity since the circling movements were dominant over all other patterns of behavior. However, histological studies revealed low activity of the prolactin cells in the anterior pituitary, suggesting that prolactin had not been secreted. Experiments with PAGE also showed that prolactin had been stored in the pituitary after apomorphine-treatment. The results obtained in group II and III, when chafing occurred at high rates, before injection with apomorphine or naloxone, suggest that the TRH sensitive neurons reacted more sensitively to a new TRH-administration. This was obviously caused by apomorphine- and naloxone-treatment.

The TRH-analog 3-Me-His^{2}-TRH increased chafing to a higher extent than TRH and it was even effective at lower concentrations. It also induced "calling," indicating that the prolactin level was quite high. "Wet-dog-shakes," which are occasionally displayed when TRH or its analog was injected, reveal that there might be interactions with the opiate-receptors, because it has been shown that the shaking syndrome can be blocked by morphine [15]. In addition, the brain areas associated with TRH-stimulated shaking, parallel sites where naloxone-induced shaking in morphine-dependent animals occurred [25]. Moreover, naloxone also elicits "wet-dog-shakes" [24]. These data suggest that more than one neuromechanism may be responsible for shaking behavior.

Since the opiate-antagonist naloxone reverses opiate-mediated actions [22], it has been widely used to identify effects elicited by opiate- or morphine-like drugs and peptides. However, it was not considered in many experiments that naloxone might cause drug-specific effects. Also, naloxone is not a pure opiate-antagonist. Naloxone suppresses actions which have not been induced by opiates [22]. It has been postulated [8] that low naloxone concentrations would reduce opiate-mediated effects caused by naloxone. According to these various effects, it is not surprising that Hemichromis reacted individually.

As has been reported, locomotor activity was reduced in naloxone-treated rats [21,26]. This was also observed in Hemichromis [2,4]. Stand digging, a characteristic of parental care, was observed less often after naloxone-treatment. This is evidence that naloxone suppresses prolactin-release, as sand digging is a characteristic of prolactin-mediated behavior. Histological studies of the anterior pituitary also showed that the activity of the prolactin cells was low after naloxone-treatment, suggesting that the secretion rate was reduced [2]. Experiments with PAGE also revealed high prolactin-concentrations in the anterior pituitary [2]. High breathing rates, which occurred shortly after naloxone administration, might be regarded as opiate antagonist effects. It has been postulated [14] that reduced breathing activity in hibernating hamsters can be reversed by naloxone. The fish also displayed vomiting and spasms of the muscles, which are char-

acteristic symptoms of opiate-deprived animals. In Hemichromis, excessive spitting, chewing, and saltatory swimming movements were observed, which might be caused by naloxone acting as an opiate-antagonist [3,4].

Hemichromis was very frightened when a fish net was presented. Since naloxone and opiates bind at the same sites, fright might have been induced by naloxone blocking binding sites which would have otherwise been occupied by endogenous peptides.

It is known that various types of opiate receptors exist. It has also been proposed that naloxone acts selectively on different types of receptors, and that naloxone may bind with non-opiate receptors. Therefore, interpretations of naloxone-effects should be carefully analyzed, as it is still doubtful on which binding sites it is most likely to act. The results indicate that most changes in Hemichromis might be signs of opiate-deprivation, although opiate-agonistic effects and interactions with ACTH should be considered.

When comparing chafing curves of a single animal (open circled curves) with average chafing curves (Figs. 1–4), there seems to be an extreme variation among measurements. This variation is due to sometimes very high chafing intensities of the single fish, often alternating with low chafing values (Figs. 1, 2 and 3). These extreme values will not always continue for the whole observation session, but these results show, how many times a fish might chafe during 5 minutes. Moreover, the rather low and more balanced average chafing rate clearly indicates that a fish does not chafe at very high rate permanently, suggesting that Hemichromis needs low chafing rates to recover. As has been shown in Fig. 2 and Fig. 4, the fish did even display quite different chafing rates when the same dose (2 μg TRH/g bw) was injected. Similar results were also observed in fighting in untreated fish or after distilled-water-injection [2]. This result reflects different chafing/fighting capabilities of the individual, which has to be investigated in further studies.

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