Differential Behavioral Effects of TFF Peptides: Injections of Synthetic TFF3 Into the Rat Amygdala

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SCHWARZBERG, H., H. KALBACHER AND W. HOFFMANN. Differential behavioral effects of TFF peptides: Injections of synthetic TFF3 into the rat amygdala. PHARMACOL BIOCHEM BEHAV 62(1) 173–178, 1999.—TFF peptides (formerly named P-domain peptides or trefoil factors) are major secretory products of mucin producing cells and are synthesized in the brain as well. Three TFF peptides have been characterized in a number of mammals including human: TFF1 (formerly pS2), TFF2 (formerly hSP), and TFF3 (formerly hP1.B/hITF). TFF1 is expressed neurally in the hippocampus, the cortex, and the cerebellum of the rat (13), as well as in cultured mouse astrocytes (14) where regulation of expression by various cytokines has been reported (12). In contrast, neural expression of TFF3 has been observed in the hypothalamus (i.e., magnocellular neurons of the supraoptic and paraventricular nuclei) and also the amygdala (28). No data are published concerning a neural expression of TFF2.

The physiological functions of TFF peptides seem to be multiple. They obviously act as motogens influencing cell migratory processes in the gastrointestinal mucosa (10,27) probably via receptors (33). They also interact with mucins, and appear as constituents of viscoelastic mucous gels. The role of TFF peptides in the central nervous system (CNS) has not yet been clarified. They could represent factors influencing the development of the CNS (13). TFF peptides could also affect behavior similar to the action of oxytocin (3,16). However, transgenic knock out mice where TFF1 or TFF3 have been deleted display no obvious neural abnormalities (19,21). The first direct studies addressing the question on behavioral effects of synthetic TFF3 in rats are presented here. Bilateral application of TFF3 into the amygdala has been chosen for system-
involving learning, memory, and anxiety (4,5,9,16, 30,35). The use of a classical passive avoidance test as well as the elevated plus-maze allow statements concerning possible effects of TFF3 on processes involving learning, memory, and anxiety (4,5,9,16, 30,35).

METHOD

Adult male Wistar rats (Harlan GmbH, Borchen, Germany) were kept under a 12 L/12 D schedule (lights on at 0600 h) with free access to standard pelleted food (Altromin GmbH, Lage, Germany) and tap water. The following experimental protocol was approved by an Institutional Review Committee for the use of animal subjects.

The animals were anesthetized with 50 mg/kg sodium pentobarbital (Sigma-Aldrich GmbH, Deisenhofen, Germany) and then placed in a stereotactic instrument. A pair of stainless steel guide cannulae of 1.0 mm o.d. and 13 mm length were introduced through small burr holes on the skull and were placed 1.0 mm above the basolateral amygdala of the left and right hemispheres. All coordinates were provided by the atlas of Paxinos and Watson (25). The cannulae were fixed chronically to the skull with dental cement (Harvard GmbH, Berlin, Germany) and acrylic (Spezialchemie, Leipzig, Germany). Cannulae were implanted in a total of 312 rats. The animals were sacrificed at the end of each experiment to verify the position of the injection; only results with the cannulae in the correct position entered the statistical evaluation.

The rats were killed by an overdose of pentobarbital. Their brains were fixed in 10% formaldehyde for at least 1 week, and the placement of the cannulae was visualized on 30-μm frozen sections after injection of methylene blue into the cannulae. Experiments were started 7 days after operation. During the recovery period, the animals were handled each day to decrease stress during the experiment.

Synthetic TFF3 based on the deduced rat sequence (28) was synthesized on a MilliGen9050 peptide synthesizer similarly as described (1,2). The experimental arrangement consisted of two “closed” (25 cm high walls) and two “open” arms (40 × 15 cm each) interconnected by a 15-cm square central area. The maze was elevated 70 cm above the floor. Light conditions were similar (30 lx) in the open and closed arms. Separate series of rats, naive to the apparatus, were placed onto the square central area facing a closed arm 1 or 24 h after the bilateral injection into the amygdala and allowed to explore the maze for 3 min. The animals showed their main locomotor activity within these 3 min. The following parameters were scored with the help of an automatic video tracking system: (a) the time spent in the different compartments of the maze, (b) the number of entries into the different compartments, and (c) the distance walked within the different compartments (measuring exactly the locomotor activity).

Analgies effects were measured in separate series 15 and 60 min after injection of TFF3 (two series with doses of 2 × 6 or 2 × 60 pg) by means of a tail-flick analgesiometer (6). Briefly, a focused light beam is directed to the tail of the rat. The rat stops light and measurement of time by a short sudden movement of the tail, if pain is perceptible. Control rats treated with 0.9% NaCl switched off the light between 7 and 10 s.

Statistical significance was evaluated by using Levene’s test for equality of variances and the two-tailed t-test (SPSS software 7.5, SPSS Inc. 1996). Differences between the TFF3-injected animals and their respective controls were considered significant if \( p < 0.05 \).

RESULTS

Passive Avoidance Tests

Passive avoidance behavior was tested after low-dose (2 × 6 pg; Fig. 1) or high-dose injections (2 × 60 pg; Fig. 2) of TFF3 into the amygdala.

Retrieval tests. The injection of TFF3 took place about 23 h after the initial foot shock, a point at which memory consolidation is not affected. Neither of the two doses significantly affected the retention trial 1 compared with the control animals treated with 0.9% NaCl. Injection of a low TFF3 dose reduced the avoidance latency significantly only in retention trials 2 and 3 (Fig. 1A). In contrast, injection of a high TFF3 dose increased the latency significantly in retention trial 2 (Fig. 2A).

Consolidation tests. Passive avoidance latency was also tested after an injection scheme typical of a consolidation test, as shown in Figs. 1B and 2B. Both TFF3 doses were injected immediately after the foot shock when memory consolidation takes place. Injection of the lower dose decreased the avoidance latency significantly in all retention trials (Fig. 1B), whereas an increase of the avoidance latencies was observed.
FIG. 1. Passive avoidance latencies after bilateral injection of 2 × 6 pg TFF3 into the rat amygdala (hatched). The results from a typical retrieval test (A) or a consolidation test (B) are shown. As a control, 0.9% NaCl was injected (dotted). The number of animals tested in each group is given above the bars. Mean values ± SE; stars indicate statistically significant p-values: A/trial 2: \( p < 0.042 (t = 2.144) \), A/trial 3: \( p < 0.01 (t = 2.944) \), B/trial 1: \( p < 0.002 (t = 3.476) \), B/trial 2: \( p < 0.012 (t = 2.700) \), B/trial 3: \( p < 0.001 (t = 3.828) \).

FIG. 2. Passive avoidance latencies after bilateral injection of 2 × 60 pg TFF3 into the rat amygdala (hatched). The results from a typical retrieval test (A) or a consolidation test (B) are shown. As a control, 0.9% NaCl was injected (dotted). The number of animals tested in each group is given above the bars. Mean values ± SE; stars indicate statistically significant p-values: A/trial 2: \( p < 0.048 (t = -2.068) \), B/trial 1: \( p < 0.036 (t = -2.231) \), B/trial 3: \( p < 0.038 (t = -2.257) \).
in most retention trials after injection of the higher dose (Fig. 2B).

**Elevated Plus-Maze Tests**

The following significant behavioral effects were observed after low-dose (2 × 6 pg; Fig. 3) or high-dose injections (2 × 60 pg; Fig. 4) of TFF3. The number of open-arm entries was not affected at both doses (data not illustrated).

Animals injected with a low dose of TFF3 showed a decreased total locomotor activity 1 h after injection when compared with control animals (Fig. 3A). However, no effect was observed concerning the activity or the time on the open arms. TFF3 at this time point apparently did not trigger mechanisms of fear (Fig. 3A). In contrast, the activity on open arms significantly increased 24 h after injection, but not the total activity (Fig. 3B). The time on open arms showed a tendency towards an increase at this time point.

Animals injected with a high dose of TFF3 could easily be distinguished by their different behavior when compared to the controls even 1 h after injection (Fig. 4A). The animals entered all arms in a manner similar to the controls but left the open arms immediately. This behavior resulted in a significantly decreased locomotor activity on open arms and a tendency towards a decrease in the time spent on open arms ($p < 0.075$) 1 h after injection. The total activity did not change in comparison to the controls at this time point (Fig. 4A). Furthermore, the open-arm activity together with the time on open arms was still reduced 24 h after injection (Fig. 4B). However, also the total activity decreased significantly at this time point.

**Platform Tests, Tail-Flick Tests**

None of the two doses applied (2 × 6 pg, 2 × 60 pg) had effects in the platform and tail-flick tests showing that motor coordination and pain sensitivity of the TFF3-treated animals were within the same range as the controls.

**DISCUSSION**

Passive avoidance tests showed maximal effects about 1 day after the application of synthetic TFF3 into the amygdala, re-

![Graphs showing activity and time on open arms](image_url)
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Regardless if a retrieval test or a consolidation test had been performed. The maximal effect was consequently at retention trial 2 in retrieval tests, whereas TFF3 was most active already at retention trial 1 in the consolidation test. One hour after application (i.e., retention trial 1 in the retrieval tests) was obviously too early to observe a significant effect. However, a tendency was visible at this time. The reason for this delay is currently not known; it is unlikely that the lag phase is due to generation of biologically active proteolytic degradation products of TFF3 because TFF peptides are remarkably stable even in the aggressive lumen of the gastrointestinal tract (11).

The different TFF3 doses injected had opposite effects in the passive avoidance tests. This was observed for both the retrieval and the consolidation test. A direct comparison of the maximal effects in the retrieval tests (i.e., retention trial 2) and the consolidation tests (i.e., retention trial 1) clearly accentuates the increased avoidance latency at the low dose ($2 \times 60$ pg), whereas the high dose ($2 \times 60$ pg) decreased the latency. A similar biphasic dose dependent effect has been described for CRH (34).

The avoidance latency reduction at the low dose of TFF3 could be due to an anxiolytic effect. This would be in line with the results from the elevated plus-maze test 24 h after injection. However, mechanisms of fear do not seem to be affected, as judged by the elevated plus-maze test results 1 h after injection of TFF3. The decrease in the total locomotor activity at this time is in agreement with a report that the amygdala generally plays an important role in the modulation of alerting mechanisms and arousal states (32).

The enhanced avoidance latency after injection of a high TFF3 dose into the amygdala might be caused by an anxiogenic effect, which is also in agreement with the elevated plus-maze test results presented. However, the effect at 24 h is correlated to a change in the total locomotor activity which can give rise to false positive results in the elevated plus-maze test (8,9,23).

In summary, injection of synthetic TFF3/monomer into the rat amygdala seems to affect anxiety bidirectionally in a dose-dependent manner. A low dose ($2 \times 6$ pg) is anxiolytic, whereas a high dose ($2 \times 60$ pg) has an anxiogenic effect, and both doses are capable of modulating total locomotor activity. Both behavioral parameters are mediated by the serotonergic transmission system in the amygdala (5,32). The basolateral nucleus, which received the TFF3 injections in the present investigations, showed the most pronounced serotonergic innervation (32).

![FIG. 4. Results from the elevated plus-maze tests 1 h (A) or 24 h (B) after bilateral injection of $2 \times 60$ pg TFF3 into the rat amygdala (hatched). As a control, 0.9% NaCl was injected (dotted). The number of animals was: (A) TFF3 $n = 12$, controls $n = 12$; (B) TFF3 $n = 13$, controls $n = 11$. Mean values ± SE; stars indicate statistically significant $p$-values.](image_url)
The passive avoidance test by itself represents a paradigm typically used for investigations of learning and memory. However, the results with the elevated plus-maze presented here do not allow a clear statement concerning the effect of TFF3 on learning and memory. Further studies necessary to clear this point will be of special interest, as the amygdala seems to represent a brain region where fear and processes of learning and memory converge (22,31).

REFERENCES