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NGF Effects on Hot Plate Behaviors in Mice

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DELLA SETA, D., L. DE ACETIS, L. ALOE AND E. ALLEVA. *NGF effects on hot plate behaviors in mice.* PHARMACOL BIOCHEM BEHAV 49(3) 701–705, 1994. – Adult CD-1 male mice were injected intravenously with 2.5 μg/g of highly purified murine NGF and then assessed for hot plate responding (52°C) at 15, 30, 60, 180, and 360 min (repeated test) or at 30, 60, or 360 min (single test, i.e., exposure to hot plate only once). Control animals received cytochrome c (2.5 μg/g). In the repeated test, NGF produced hyperalgesia, increasing the number of jumps (particularly at 30 and 60 min postinjection), while in the single test the pain reaction of NGF-treated animals remained unaffected. Sensitization of C-fibers in the periphery or release of bioactive mediators from mast cells may account for NGF-induced changes in nociception.

Nerve growth factor Mouse Hot plate Pain Hyperalgesia Behavior

NERVE growth factor (NGF) is a neurotrophic factor that exerts a survival and trophic effect on peripheral sympathetic and sensory neurons and other neural crest-derived cells (17,18). Although the trophic and differentiative effects of NGF on developing peripheral and central neurons have been extensively studied in the past (13,17,31), only recently has the possible role of NGF in adult neurobehavioral regulations been thoroughly investigated (1–3,15–17,26).

Since our first report (19), NGF has been shown to be implicated in nociception (20,21,30). Lewin and co-workers reported that, in adult rats, NGF-induced mechanical and heat hyperalgesia appear to be due to two different mechanisms. The mechanical hyperalgesia may be due to a central effect, whereas the heat hyperalgesia is likely to result, at least in part, from the sensitization of peripheral receptors to heat. Although the role of NGF is known to change as the animal matures, its effect on peripheral sensory innervation and the interaction with specific nerve endings is maintained through adulthood, acting as a link between inflammation and hyperalgesia (19,20). More recently, Mobley et al. (12,30) described the amino-terminal NGF octapeptide (NGF-OP), which given as an intradermal injection acts as a hyperalgesic agent capable of altering the pain threshold in injured target regions of NGF-responsive neurons.

Previous studies, performed on rats, evaluated NGF- or NGF-OP-induced changes in nociception, measuring mechanical thresholds either with Von Frey hairs (21) or Randall-Selitto paw-withdrawal test (30), while thermal hyperalgesia was assessed by foot withdrawal response from water at 49°C (21). All these studies employed tests in which subjects were restrained during pain evaluation, and restraint has been shown to modify a variety of physiological and behavioral parameters, resulting in alteration of pain perception (26). However, the Argreaves method (14), measuring a more conditionated behavior than foot or tail flick, also reveald NGF-induced hyperalgesia in unrestrained rats (27).

In the present study we investigated in CD-1 mice the effects of NGF on hot-plate responding, using a test that evaluates complex behavioral responses to a noxious thermal stimulus (52°C) in unrestrained animals. Moreover, behavioral performances in the hot plate test allows to individuate different neural subsystems, specifically controlling pain reactivity (6). Because in previous observations we found NGF-induced hyperalgesia only after repeated hot-plate assessments (19), we confronted single testing with five repeated exposures to the hot plate.

METHOD

Animals and Treatment

Male outbred albino Swiss (CD-1) mice (mean weight 41 g) purchased from Charles River Italia (Calco, Italy) were used. They were kept in an air-conditioned room at 21 ± 1°C and 60 ± 10% relative humidity, with a 12/12 red light/white light cycle (red light on at 0930 h). Pellet food (enriched standard diet purchased from Piccioni, Brescia, Italy) and tap

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water were continuously available. Ninety sexually experienced subjects were individually housed for 2 weeks in Plexiglas boxes (33 × 13 × 14 cm) with metal tops and sawdust as bedding. In both experiments (repeated and single tests) mice were randomly assigned to groups of 10 subjects each. Experimental groups were treated either with NGF (Chemicon, Temecula, USA) at 2.5 μg/g or cytochrome c (No. C-7752, Type VI, Sigma, St. Louis, MO; same dose; CYT group). This dose was selected after a pilot study showing that a lower dose of NGF (1μg/g) did not exert significant effects. NGF was prepared following the method of Bocchini and Angeletti (8) and further purified to eliminate renin-like activity by additional carboxymethyl-cellulose chromatographies, as suggested by Cozzari et al. (9). Cytochrome *c* is generally employed as a control treatment for its physicochemical characteristics, which are similar to those of the NGF molecule, but lack NGF neurotrophic activity. Treated animals received an intravenous (IV) injection before the test. Each animal was tested only once. Procedures were discussed with the local ethical committee.

Hot Plate Test

Each subject was placed in the center of a glass cylinder-covered (diameter 19 cm) hot plate apparatus (model D837 Socrel, Comerio, Italy) maintained at 52 ± 0.1°C. Nociceptive heat sensitivity was assessed by scoring latency time to first licking and jumping. The number of licking episodes were also recorded for both fore- and hindlimbs. Cutoff time was 1 min. Behavior was video recorded using a professional Sony Videocassette Recorder V0-5800PS apparatus; scoring was carried out using an Esterline Angus Operation Recorder (A620X model, trans. type), latency time was determined to the nearest millisecond by a digital stopwatch.

Procedure

In the first experiment (repeated test), each animal was injected once with either NGF or CYT and then tested on the hot plate 15, 30, 60, 180, and 360 min after the injection. In the second experiment (single test), each animal was injected with NGF or CYT and then tested on the hot plate either at 30, 60, or 360 min after injection.

Statistical Analysis

Data were analyzed by parametric analyses of variance (ANOVA) with two levels of treatment as between-subject factor.

RESULTS

In the repeated hot plate test, NGF-treated animals showed increased nociceptive responding when compared to cytochrome-treated mice (see Fig. 1). In particular, the number of jumps was significantly higher than in controls, $F(1, 18) = 4.56$, $p \leq 0.05$, and latency to the first jump was shorter in NGF-treated mice [just missed statistical significance, $F(1, 18) = 3.91$, $p \leq 0.06$]. Such an hyperalgesic profile emerged 30 min after injection, peaked at 60 min, and decreased slowly in the two subsequent assessments. The level of jumping response in control mice remained fairly stable over time, appearing only in the second exposure to the hot plate. In no case did NGF exert a significant effect on latencies or number of either forelimb or hindlimb licking response (Table 1a). It should be noted that no visible sign of skin inflammation was observed after the hot plate test.

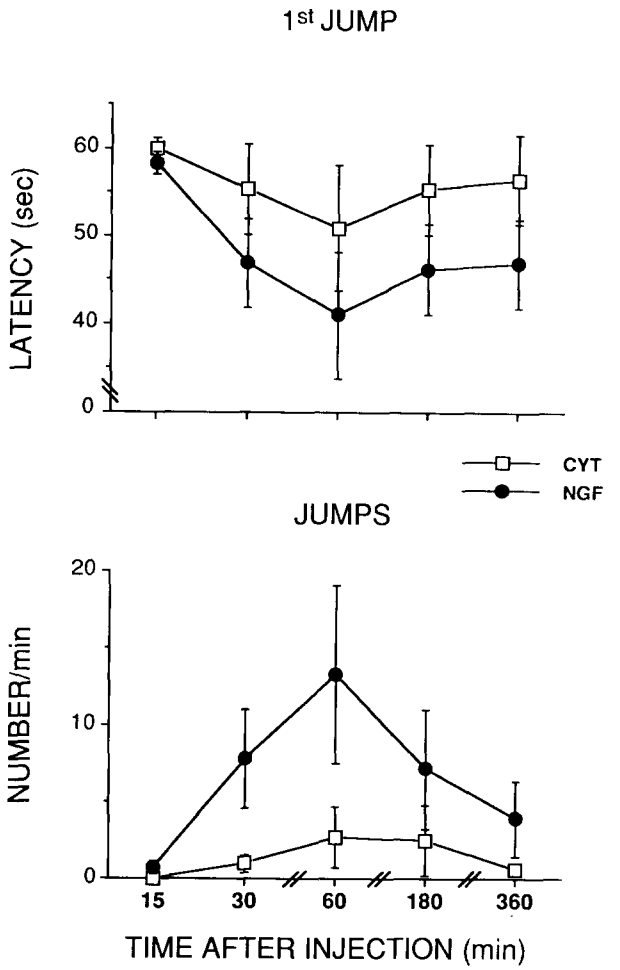

FIG. 1. Hot plate jumping response of male mice injected IV with either murine NGF (2.5 μg/g) or cytochrome c (CYT, 2.5 μg/g) in repeated tests (n = 10 animals/group). Data represent mean levels ± SEM.

In the case of single tests, no significant effect of NGF treatment was evident for any of the measures considered (Table 1b). The latency of forelimb and hindlimb licking at 30, 60, and 360 min, as well as the absence of jumping, were very similar to the corresponding levels recorded at 15 min in the repeated test. The number of NGF-treated animals displaying a jumping response in repeated test at 30, ,60 and 360 min was significantly higher than for animals of the same group in the single test (p = 0.016, Fisher's exact probability test).

DISCUSSION

Previous studies on thermal hyperalgesia of adult rats showed that, 15 min after a single NGF injection, the latency for foot withdrawal from a 49°C water bath was significantly reduced (21), compared with the same animals tested just before the injection. Our data obtained with mice confirmed that NGF administration enhances heat hyperalgesic response, but only in the case of the repeated test. In fact, NGF-treated animals jumped more than controls and showed a shorter latency to the first jump when tested at 30 and 60 min after NGF injection following a test at 15 min.

A fine-grain analysis of the behavioral performance of the

TABLE 1

NGF EFFECTS ON HOT PLATE RESPONDING

	CYT					NGF				
Time after Injection (Min)	15	30	60	180	360	15	30	60	180	360
					(a) Repeated Test					
Latency forelimb licking	17.6 ± 2.4	22.4 ± 4.8	20.0 ± 3.2	26.2 ± 6.2	29.6 ± 3.3	26.0 ± 5.7	36.0 ± 7.6	38.7 ± 6.7	29.8 ± 5.8	24.1 ± 4.8
Latency hindlimb licking	33.2 ± 4.3	28.9 ± 6.2	26.8 ± 4.0	47.6 ± 5.8	46.7 ± 3.0	31.4 ± 2.5	35.5 ± 6.7	31.3 ± 7.0	34.8 ± 6.3	37.5 ± 5.3
Latency jumping	60.0 ± 0.0	55.3 ± 2.7	50.9 ± 4.8	55.3 ± 3.6	56.5 ± 1.8	58.3 ± 1.2	46.9 ± 5.1	41.0 ± 7.2	46.2 ± 5.2	46.9 ± 5.1
No. forelimb licking	6.0 ± 1.1	4.4 ± 0.7	4.3 ± 0.9	4.5 ± 1.0	3.9 ± 0.8	4.3 ± 1.0	2.0 ± 0.7	2.6 ± 0.9	4.0 ± 0.7	4.9 ± 0.8
No. hindlimb licking	5.0 ± 1.2	5.3 ± 1.4	4.5 ± 1.1	2.4 ± 1.1	1.9 ± 0.5	5.1 ± 0.7	3.3 ± 1.1	4.7 ± 1.5	3.1 ± 1.2	3.1 ± 0.9
No. of jumps	0.0 ± 0.0	1.0 ± 0.5	2.7 ± 2.0	2.5 ± 2.3	0.6 ± 0.3	0.7 ± 0.4	7.8 ± 3.2	13.3 ± 5.8	7.1 ± 3.9	3.9 ± 2.4

	CYT			NGF		
Time after Injection (Min)	30	60	360	30	60	360
		(b) Single Test				
Latency forelimb licking	16.4 ± 1.8	15.1 ± 1.7	17.9 ± 1.9	14.7 ± 1.7	15.4 ± 3.2	15.0 ± 1.8
Latency hindlimb licking	29.2 ± 2.2	24.4 ± 3.0	30.6 ± 3.7	34.6 ± 4.7	24.9 ± 2.4	25.7 ± 1.2
Latency jumping	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0
No. forelimb licking	6.3 ± 0.0	6.9 ± 0.7	6.9 ± 1.3	6.2 ± 1.1	7.5 ± 1.2	7.5 ± 0.7
No. hindlimb licking	6.1 ± 1.0	7.8 ± 0.8	6.2 ± 1.0	4.6 ± 1.1	5.5 ± 0.7	7.2 ± 1.0
No. of jumps	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Data are mean levels of 10 male mice for each group (± SE). Times are in seconds.

NGF-treated animals showed a selective increase in jumping, while both fore- and hindlimb licking remained unaffected. The emergence of a jumping response only at the second assessment can be explained as an attempt to avoid actual paw contact with the heated surface, a reduction of exploratory behavior in a more familiar environment, or both. Moreover, it has been previously suggested that in the hot plate test, the paw licking response (short latency) represents the sensory component of pain, while the jumping response (long latency) may represent the affective component of pain (6). The latter includes stress-induced changes in emotional tone affecting pain reactivity, such as release of endorphins and enkephalins, which act as modulators of limbic mechanisms involved in the elaboration of emotions (5,6,24). The sensory and affective components of pain can be selectively modified, as shown for instance for endogenous opioid ligands, which increase jumping from a hot plate in rats while leaving paw licking response unaffected (6).

Our results are in agreement with a recent study showing that bradykinin-induced hyperalgesia was observed only after repeated testing (29). Moreover, the fact that we observed significant differences after repeated testing could be indicative of an enhanced responsiveness due to a decreased threshold to heat possibly caused by sensitization of peripheral C-fibers (20). However, at the level of single C-fibers this sensitization (25) rarely lasts as long as the intertest periods used in our experiment. It seems more likely that the lack of result with the single test protocol is due to the above-mentioned training effect. Furthermore, it has been shown that NGF-OP produces a decrease in nociceptive threshold only when the skin has been traumatized by prior application of chloroform (30).

Biochemical data suggest that kinins produced locally in injured skin contribute to inflammation. It is possible that NGF produced or released in injured tissue participates in the hyperalgesia by serving as substrate of kinins (23). In addition, previous studies have shown that NGF is a potent stimulator of degranulation of mast cells, releasing 5-hydroxytryptamine and histamine, which are involved in hypersensitivity reactions (7). It is possible that circulating NGF induces release of bioactive mediators from mast cells localized in close apposition to peripheral cutaneus sensory nerve endings, thereby facilitating nerve activity, e.g., by increasing substance P levels (4,10,11,22). A preliminary study, indicating that after mast cell degranulation rats do not display heat hyperalgesia following treatment with NGF, seems to favour this latter hypothesis (27).

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