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Tolerance to Tobacco Smoke- and Nicotine-Induced Analgesia in Rats

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MOUSA, S. A., V. J. ALOYO AND G. R. VAN LOON. *Tolerance to tobacco smoke- and nicotine-induced analgesia in rats.* PHARMACOL BIOCHEM BEHAV 31(2) 265–268, 1988.—Acute exposure of male Sprague-Dawley rats to either nicotine or tobacco smoke results in analgesia as measured by tail-flick latencies. A second treatment, 24 hr after the first, failed to produce analgesia, thereby demonstrating the rapid development of tolerance. The restraint which was a necessary part of the tobacco smoke exposure also produced analgesia, although of a more transient nature and lesser magnitude than that resulting from tobacco smoke exposure. Tolerance also developed to restraint stress-induced analgesia. The long-term (43 weeks) daily exposure of rats to tobacco smoke or restraint stress resulted in the development of cross-tolerance, suggesting that these two procedures share, at least in part, a common mechanism. Additionally, long-term tobacco smoke exposure resulted in an increased tail-flick latency when the animals had been withdrawn from tobacco smoke for 24 hr, suggesting the development of tolerance. The data also suggest a differential time course for the development of tolerance and dependence. This is the first report that addresses the effect of acute and chronic tobacco smoke exposure on pain sensitivity.

Analgesia Tobacco smoke Nicotine stress Tolerance

MANY psychological and physiological effects of tobacco smoke have been investigatged and have been attributed to the nicotine content of the smoke (2,6). Nicotine has been shown to produce antinociceptive effects in a variety of species (10,13). Nicotine produces changes in behavioral responses such as spontaneous motor activity, central depression and learning. Nicotine's effects on these behaviors is characterized by the rapid development of tolerance (7, 8, 12). The mechanism(s) underlying the tolerance to repeated administration of nicotine remain to be elucidated.

In this report we have compared the analgesic effect of acute nicotine and tobacco smoke and the development of tolerance to repeated administration. Furthermore, we examined the effects of long-term daily tobacco smoke exposure on pain sensitivity.

METHOD

Adult male Sprague-Dawley rats were used in all experiments.

Tobacco smoke exposure was performed at the Tobacco and Health Research Institute, University of Kentucky, using their standard procedure. In this procedure, rats are restrained for 10 min during each smoke exposure session. A 2RI cigarette (University of Kentucky Reference Cigarette) containing 2.65 mg nicotine was machine-smoked at a rate of one puff per min. Sham animals were restrained and handled identically except that they were exposed to puffs of air instead of tobacco smoke.

Pain sensitivity was determined by measuring tail-flick latency by a modification (9) of the method of D'Amour and Smith (3). A 5 mm diameter beam of light from a 600 W tungsten halogen lamp (Sylvania SVY) was projected 1.5 cm onto the rat's tail. The time until flicking of the tail (latency) was recorded to the nearest 0.01 sec. A 10 sec maximum exposure to the light was chosen in order to avoid damage to the rat's tail. Light intensity was controlled by regulating lamp voltage, but within each experiment lamp voltage was held constant in order to achieve an average basal latency of 5 sec. Rats were adapted to the tail-flick apparatus and attendant handling procedures for four successive days immediately prior to actual data collection. This adaptation procedure results in a more reproducible and stable baseline (9). Repetitive testing was performed at 3 min intervals in order to examine the time course of treatment effects.

Experimental Design

Experiment 1. Effect of daily nicotine treatment on tail-flick latencies. Naive rats were adapted to the pain sensitivity testing protocol for four days. On the fifth day, basal tail-flick latencies were measured immediately before and 3, 6 and 9 min following the subcutaneous injection of nicotine (1 mg/kg; Kodak) diluted in physiological saline. Control animals received an injection of saline. Twenty-four hours later, pain sensitivity was again measured immediately preceding and subsequent to a second injection of nicotine or saline.

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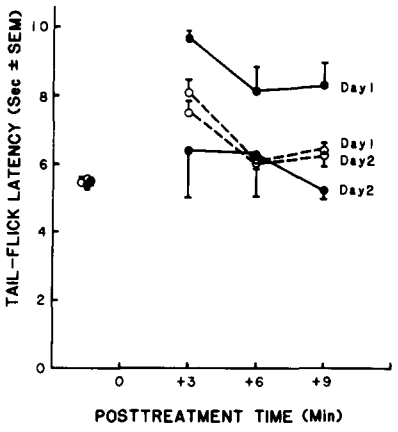

FIG. 1. Effect of daily nicotine treatment on tail-flick latency. Tail-flick latencies were measured immediately before (left) and 3, 6 and 9 min after the subcutaneous injection of nicotine (●) or saline (○) on the first and second days. Nicotine significantly ($p<0.03$) increased the tail-flick latencies relative to saline treatment on the first exposure but not on the second. The symbols represent the means and the bars the SEM for 6 rats.

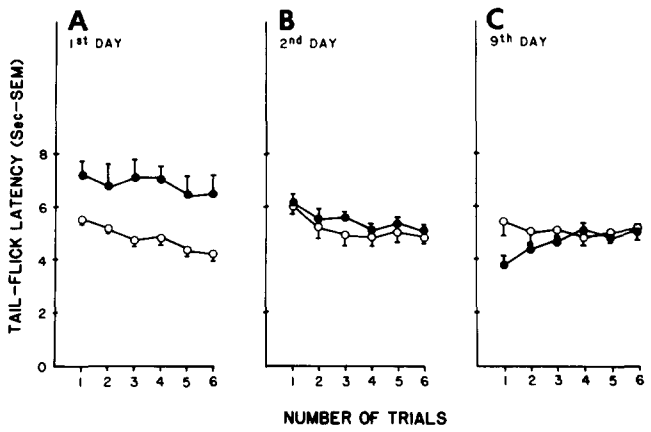

FIG. 2. Effect of daily tobacco smoke exposure on tail-flick latencies. Tail-flick latencies were measured immediately before (○) and after (●) the first, second and ninth daily tobacco smoke exposure. Only after the first day did tobacco smoke exposure significantly increase tail-flick latencies ($p<0.006$). The latencies measured immediately before smoke exposure did not significantly vary with days of treatment. The results are the means (±SEM) for 8 rats.

Experiment 2. Effect of daily tobacco smoke exposure on tail-flick latencies. Naive rats were adapted to the pain sensitivity testing procedure for 4 days. On the fifth and subsequent days these rats were subjected to tobacco smoke exposure. Tail-flick latencies were measured immediately preceding and following the daily tobacco smoke exposure on the first, second and ninth days.

Experiment 3. Effect of acute tobacco smoke exposure on chronically smoke-exposed animals. Groups of age-matched rats were subjected to one of the following treatments for 43 weeks: chronic daily tobacco smoke exposure, chronic daily sham smoke exposure or no treatment (naive). During the 43rd week, the rats were adapted to the pain sensitivity procedure for 4 days. On the day of the experiment, each rat was subjected to the pain sensitivity testing protocol four times in succession. The basal (basal 1) tail-flick latency was measured approximately 24 hr after the last treatment. Immediately upon completion, the rats were stressed by being subjected to the sham smoke exposure procedure. Within 3 min following stress, pain sensitivity testing was initiated. Subsequently, the rats were returned to their home cage for 2 hr. A second basal latency (basal 2) was then determined, followed immediately by tobacco smoke exposure. Within 3 min, pain sensitivity testing was initiated.

Statistical Analysis

Data were analyzed using ANOVA for repeated measures and individual comparisons based on Tukey's HSD procedure.

RESULTS

Experiment 1

Effect of daily nicotine treatment on tail-flick latencies. The basal latency in naive rats ranged from 5 to 6 sec. Saline injection resulted in a transient elevation in tail-flick latency so that 3 min after injection the latency was approximately 8 sec (Fig. 1). However, by 6 min the latency of saline-treated animals had returned to basal levels. In contrast, nicotine injection resulted in a significant elevation of tail-flick latency ($p<0.03$) which was maintained throughout the 3 testing trials. When tested 24 hr later the tail-flick latencies for both groups had returned to pretreatment values (5 to 6 sec). A second saline injection resulted in a transient increase in tail-flick latency nearly identical to that observed the first day. However, the second nicotine administration, 24 hr after the first, failed to significantly alter the tail-flick latency (Fig. 1) indicating the development of tolerance.

Experiment 2

Effect of daily tobacco smoke exposure on tail-flick latency. The basal (pretobacco smoke exposure) tail-flick latencies of naive rats were approximately 4 to 5 sec. The first tobacco smoke exposure significantly elevated the tail-flick latency to approximately 7 sec throughout the 6 testing trails (Fig. 2A). However, when these animals were retested approximately 24 hr later, the basal tail-flick latencies had returned to presmoke exposure levels (Fig. 2B). In marked contrast to the first tobacco smoke exposure, the second exposure failed to significantly alter the tail-flick latencies, indicating the development of tolerance. On the 9th day (after daily smoke exposure for 8 days) the basal tail-flick latencies were still at the presmoke exposure levels observed on day 1. The 9th smoke exposure, in contrast to the first and second, resulted in a transient decrease in latency (Fig. 2C).

Experiment 3

Effect of acute tobacco smoke exposure in chronically smoke-exposed animals. The basal tail-flick latency of naive rats was approximately 6 sec (Fig. 3A). The acute stress of the sham smoke exposure procedure significantly elevated the latency. The average latency 3 min after this treatment was greater than 8 sec. The effect of acute stress was transient, the latency exhibiting a rapid decline to basal levels within 15 min. Similarly, acute tobacco smoke

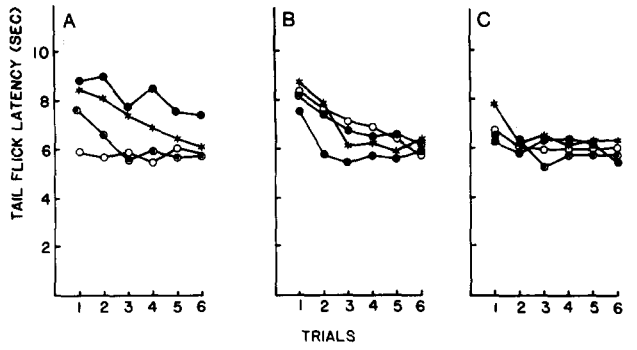

FIG. 3. Effect of acute tobacco smoke-exposure in chronically smoke-exposed animals. Naive (A), chronically smoke-exposed (B) and chronically sham smoke-exposed (C) rats were sequentially subjected to acute stress (sham-smoke exposure) and acute tobacco smoke exposure. Tail-flick latencies were measured immediately before (basal 1, ⊙) and after (*) acute stress. After a two hour recovery period the latencies were again measured immediately before (basal 2, ○) and after (●) smoke exposure. The results represent the mean of 5 to 6 rats.

exposure resulted in an elevated latency of nearly 9 sec at 3 min after treatment. However, in contrast with the effect of acute stress, the present treatment resulted in an elevated latency throughout the 18 min of testing. Tobacco smoke-induced analgesia was of a significantly greater magnitude that restraint stress-induced analgesia ($p<0.002$).

Animals subjected to chronic stress (sham smoke exposure) for 43 weeks exhibited a basal latency equivalent to that observed in naive rats. However, neither acute stress (sham smoke exposure) nor tobacco smoke exposure resulted in an altered tail-flick latency. Tail-flick latencies after both treatments remained stable at approximately 6 sec (Fig. 3C). These results suggest that chronic stress exposure results in tolerance to stress-induced analgesia and in cross-tolerance to tobacco smoke induced analgesia.

Rats chronically exposed to tobacco smoke differed from naive animals in several respects. The pretreatment (basal 1) latencies obtained approximately 24 hr after the previous smoke exposure were significantly elevated relative to naive rats (Fig. 3B,C). The first tail-flick trial with these chronically smoke-exposed animals resulted in a latency of 8 sec (Fig. 3B). This elevated latency declined to approximately 6 sec by the 6th testing trial. Furthermore, acute stress (sham smoke exposure) did not further elevate the tail-flick latencies relative to pretreatment values. These data further support the hypothesis of cross-tolerance between tobacco smoke- and stress-induced analgesia. Two hours after stress treatment basal latencies (basal 2) were nearly identical to those observed in basal 1. Tobacco smoke treatment of these chronically smoke-exposed rats not only failed to elevate the tail-flick latency but actually resulted in a significant decrease in latency as compared to pretreatment (basal 2) levels. The postsmoke exposure latencies were approximately equal to the basal latencies observed in naive or chronically stressed rats.

DISCUSSION

Nicotine administration, tobacco smoke exposure and restraint stress all acutely resulted in decreased pain sensitivity in previously untreated rats. The analgesia induced by acute tobacco smoke exposure was greater than that elicited by the restraint procedure which was a necessary part of the smoke exposure procedure. Thus it is clear that tobacco smoke itself results in increased tail-flick latency. Tobacco smoke-induced analgesia is presumably due to the nicotine content of the smoke. This suggestion is supported by the similar production of analgesia in naive rats following acute nicotine treatment or tobacco smoke exposure and the rapid development of tolerance to the analgesic effects of both treatments.

Rapid development of tolerance to nicotine's effect has been previously reported by Domino (4). He reported a partial attenuation of the acute nicotine-induced decrease in avoidance behavior upon a second injection of nicotine. In our study, continued daily tobacco smoke exposure maintained the tolerance to tobacco smoke-induced analgesia throughout the testing period for at least 43 weeks. The results of other experimenters (5, 11, 12) suggest that continued tobacco smoke exposure may indeed be unnecessary for the maintenance of tolerance. Stolerman and co-workers (11,12) reported that tolerance to a nicotine-induced decrease in spontaneous motor activity was maintained for at least 80 days after cessation of nicotine administration. Likewise, Falkeborn and colleagues (5) reported tolerance to nicotine's modification of several behavioral parameters after a single dose of nicotine. This tolerance was maintained for at least 31 days after the withdrawal of nicotine.

The mechanism for the rapid development and maintenance of tolerance to nicotine is still unclear. One may speculate that the nicotine-induced release of an endogenous mediator becomes desensitized. Equally plausible is the hypothesis that repeated nicotine treatment results in the uncoupling of the affected receptors and neuromodulators. In any event, the short half-life of nicotine coupled with the maintained tolerance even after cessation of nicotine administration is a strong indication of an indirect mechanism. Chronic restraint stress produced tolerance to both restraint-induced and tobacco smoke-induced analgesia. Similarly, acute restraint in chronically smoke-exposed rats did not alter tail-flick latency. This cross-tolerance suggests that restraint stress- and tobacco smoke-induced analgesia may share, at least in part, a common mechanism of action.

Long-term daily tobacco smoke exposure not only maintains tolerance to smoke-induced analgesia, but also alters the basal pain sensitivity state of the animal. After 43 weeks of daily tobacco smoke exposure, an increased basal tail-flick latency of the rats was observed when the animals were withdrawn for 24 hr. This change may be due to a nicotine-induced state of dependence. The time course of development of tolerance and development of dependence differ greatly. Tolerance was observed 24 hr after a single tobacco smoke exposure, whereas dependence was not yet observed after 9 daily exposures. Thus this withdrawal effect develops after long-term exposure to tobacco smoke, suggesting that differing mechanisms underlie these phenomena.

This tolerance to nicotine-induced analgesia, together with the withdrawal-induced analgesia, may play a role in maintaining smoking behavior.

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