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BRIEF COMMUNICATION

Effect of pCPA on Nicotine-Induced Analgesia

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COOLEY, J. E., G. A. VILLAROSA, T. W. LOMBARDO, R. A. MOSS, S. C. FOWLER AND S. SULT. *Effect of pCPA on nicotine-induced analgesia.* PHARMACOL BIOCHEM BEHAV **36**(2) 413–415, 1990.—This study investigated the effect of para-chlorophenylalanine (pCPA), on nicotine-induced analgesia. pCPA reduces physiological levels of 5-HT, a neurotransmitter that has been linked to pain. The effects of naloxone HCl and mecamylamine HCl on this analgesia were also assessed. Subjects were 24 albino rats. Each group of eight rats was injected subcutaneously (SC) with nicotine sulphate, followed by an intraperitoneal (IP) injection of one of the potential antagonists. Behavioral analgesia was assessed using the tail-flick test. Data analysis revealed that pCPA did not affect nicotine-induced analgesia. Consistent with past research, naloxone also had no effect, and mecamylamine effectively eliminated this analgesia. The results are interpreted in light of current knowledge of this behavioral analgesia and pain perception, in general.

Nicotine pCPA Naloxone Mecamylamine Tail-flick test Pain Analgesia

ADMINISTRATION of nicotine to laboratory animals has been associated with decreased responsivity to thermal stimuli. This behavioral analgesia was first demonstrated in white mice using the hot-plate test (6). Similar results have been obtained in both rats and mice using the tail-flick test (8,11).

Mecamylamine HCl, a centrally active cholinergic antagonist, effectively blocks nicotine-induced analgesia. Conversely, hexamethonium, an antagonist at peripheral nicotinic receptors, has no effect on this analgesia (7, 8, 11). Based on these data, the site of this effect of nicotine appears to be the CNS.

However, the physiological basis of nicotine-induced analgesia remains unclear. Scopolamine HCl, a muscarinic antagonist, has been reported to both block (8) and not block (12) nicotine-induced analgesia. Naloxone HCl has also failed to block this analgesia in the two studies investigating the role of endogenous opiates in this phenomenon (8,11).

The purpose of the present study was to investigate whether alterations in 5-hydroxytryptamine (5-HT), through synthesis inhibition, would affect nicotine-induced analgesia. 5-HT has been implicated in both pain modulation (1) and the action of nicotine (9). Furthermore, rats injected with 0.8 mg/kg nicotine sulphate (expressed as the salt) in preliminary work in our laboratory (unpublished results) demonstrated Straub tail, a behavior associated with 5-HT (5). Based on these data, it was predicted that depletion of 5-HT, by administration of pCPA, would result in reduction of the behavioral analgesia associated with nicotine. Two additional groups of rats were treated with naloxone HCl and mecamylamine HCl. Consistent with past research, it was predicted that naloxone would have no effect, whereas mecamylamine would eliminate the behavioral analgesia associated with nicotine administration.

METHOD

Subjects

Subjects were 24 male Sprague-Dawley rats (Holtzmann Co.), 150 to 200 days old and weighing between 380 and 500 grams. They were housed in individual wire-mesh cages and had free access to food and water. A constant temperature and light/dark cycle (12 hr) were maintained in the room in which they were housed. Subjects were divided into three groups of eight rats each. Each group was treated with one of the three putative antagonists.

Procedure

Behavioral analgesia was assessed using a standard tail-flick

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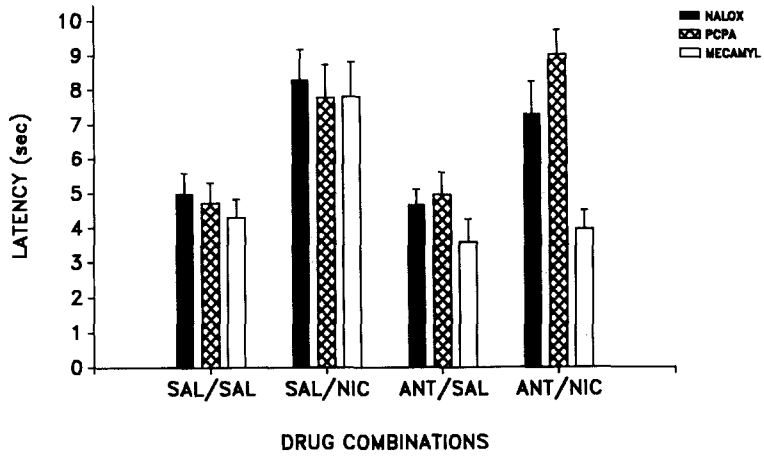

FIG. 1. Tail-flick latencies for each group after administration of each drug combination (SAL/SAL = saline/saline; SAL/NIC = saline/nicotine; ANT/SAL = antagonist/saline; ANT/NIC = antagonist/nicotine).

apparatus (3). A series of ten tail-flick trials were conducted at two-minute intervals, beginning two minutes after the nicotine or saline injection. To prevent tissue damage by the radiant stimulus, trials were terminated after ten seconds.

The same method of determining the potential antagonist's effect was used with each group. The antagonist, or an equal volume of saline, was injected intraperitoneally (IP). A 0.8 mg/kg dosage of nicotine, or an equal volume of saline, was then injected subcutaneously (SC). This dosage of nicotine was found to induce behavioral analgesia lasting a minimum of ten minutes in our preliminary research.

Rats were tested under four experimental conditions: IP saline, SC saline; IP saline, SC nicotine; IP antagonist, SC saline; and IP antagonist, SC nicotine. Naloxone and mecamylamine reach full effect in 20 minutes, and this was the amount of time between IP and SC injections in these two groups. A 72-hour interval was required in the pCPA group, in order to ensure an expected 85% depletion of 5-HT (2). A 16-day interval was required between the IP injections of pCPA and saline to ensure that the animal's 5-HT level had returned to normal. Because of this long-term depletion, animals in this group received only one IP injection for each of the two SC conditions. The intervals between IP injections in the naloxone and mecamylamine groups were, respectively, 24 and 48 hours. Because these substances' effects are relatively transient, four IP injections were administered. The design of the study was such that the order effects were completely counterbalanced. Thus, the potential effect of order of treatment was eliminated. The study was completed over an 18-day period.

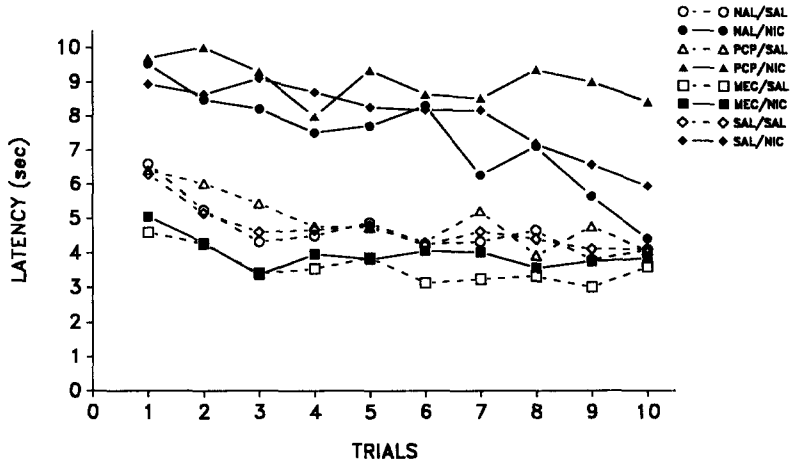

FIG. 2. Tail-flick latencies across trials for intraperitoneally injected saline and each of the three antagonists, naloxone, pCPA, and mecamylamine, in combination with subcutaneously administered saline (open symbols and dashed lines) or nicotine (solid symbols and lines). NAL/SAL = IP naloxone and SC saline; NAL/NIC = IP naloxone and SC nicotine; PCP/SAL = IP pCPA and SC saline; PCP/NIC = IP pCPA and SC nicotine; MEC/SAL = IP mecamylamine and SC saline; MEC/NIC = IP mecamylamine and SC nicotine; SAL/SAL = IP saline and SC saline; SAL/NIC = IP saline and SC nicotine.

RESULTS

A $3 \times 4 \times 10$ repeated measures analysis of variance (ANOVA) was used to examine the data. The between-subjects factor was group (naloxone, pCPA, or mecamylamine), and the within-subjects factors were drug combination [IP saline, SC saline (SAL/SAL); IP saline, SC nicotine (SAL/NIC); IP antagonist, SC saline (ANT/SAL); and IP antagonist, SC nicotine (ANT/NIC)] and trials (1 to 10). Due to procedural difficulties, the data from one subject receiving mecamylamine were excluded.

Significant main effects were obtained for group, $F(2,20) = 14.59$, $p<0.001$, and drug combination, $F(3,60) = 54.50$, $p< 0.001$, as well as a significant interaction between these two factors, $F(6,60) = 8.04$, $p<0.001$. The interaction is depicted in Fig. 1. Mean tail-flick latencies were longer for all groups after administration of the SAL/NIC injections (7.96 seconds), as compared to the SAL/SAL control injections (4.68 seconds).

Administration of the antagonist prior to the nicotine injection had significantly different effects in each of the groups. Specifically, post hoc analyses (Tukey) showed that the mean latency in the mecamylamine group after the ANT/NIC injections (3.97 seconds) was significantly shorter than the mean latency in the pCPA group after the ANT/NIC injections (9.02 seconds). The mean latency of rats in the naloxone group after the ANT/NIC injections (7.31 seconds) did not differ significantly from either of the other two groups. No other significant differences among the group $\times$ drug combination cells were found.

Figure 2 illustrates the significant reduction in tail-flick latencies across time, trial effect, $F(9,180) = 17.19$, $p<0.001$. No interaction of trials with group or drug combination was significant.

DISCUSSION

The failure of pCPA to antagonize the behavioral analgesia associated with nicotine administration suggests that 5-HT does not play a role in this phenomenon. Congruent with past research (8,11), naloxone also was ineffective in blocking this action of nicotine, thereby indicating that opioid peptides also probably are not involved. Finally, as expected (7, 8, 11), mecamylamine effectively blocked the analgesic effects of nicotine, suggesting that central nicotinic receptors are the primary mediators of this phenomenon.

The lack of blocking by both naloxone and pCPA may be interpreted in light of evidence that opioid peptides and 5-HT may inhibit pain transmission through many of the same CNS pain pathways (4). Thus, a clear aim of future research should be the exploration of other pain pathways, not linked to either of these putative neurotransmitters. For instance, several studies (12) have demonstrated the existence of a distinct norepinephrine pathway contributing to behavioral analgesia. Since yohimbine has been shown to block the analgesic effect of nicotine in mice and rats (11), it appears that norepinephrine receptors may play an important role in this phenomenon. Further research is needed to explore this hypothesis.

ACKNOWLEDGEMENT

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