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BRIEF COMMUNICATION

Hypoalgesia Induced by Counter-Irritation Is Not Affected by pCPA Pretreatment

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CALVINO, B. *Hypoalgesia induced by counter-irritation is not affected by pCPA pretreatment.* PHARMACOL BIOCHEM BEHAV **35**(3) 731–734, 1990.—In a previous work (4), it has been described that a noxious visceral stimulation through the intraperitoneal injection of acetic acid (ipAA) induced a transient and low magnitude increase in tail-flick latencies, but a marked increase in the threshold for vocalization and hot-plate latencies. In the present work, this phenomenon of hypoalgesia through counter-irritation was investigated in intact rats with or without pretreatment with the potent serotonin depletor parachlorophenylalanine (pCPA). Three behavioural tests were performed. In two tests (tail flick, vocalization induced by transcutaneous electrical stimulation of the tail), pCPA pretreatment induced an increase of baseline levels, before IP injection of the algogenic agent (ipAA). In the third test, pCPA pretreatment had no effects on jump latencies. Parachlorophenylalanine pretreatment had no effect upon hypoalgesic actions of IP injected AA in all three tests. These results are discussed in terms of pCPA's differential effects upon basal nociception and analgesia induced by various heterotopic nociceptive stimulations.

Pain Analgesia Counter-irritation Parachlorophenylalanine Serotonin depletion

COUNTER-IRRITATION, i.e., the paradoxical pain relieving effect of pain elicited from heterotopic body areas, have been known since antiquity and various non-Western (e.g., Chinese) medical procedures still include counter-irritation as a pain-relieving technique. In fact, old "popular" methods of medical practice included the therapeutic use of counter-irritation. Counter-irritation is a very large and complex phenomenon including modulation of the inflammatory response as well as pain relieving effects [see reviews in (2) and (8)].

The first experimental investigation of counter-irritation in man was made by Duncker (5). He undertook "experiments on the mutual influence of pains" and noticed that without exception, an "active" pain (A) induced a decrease of a distant and simultaneous "passive" pain (P) if A was stronger than P and, if so, in proportion to A's relative intensity.

In an earlier report (4), it has been established in the rat that the intraperitoneal injection of acetic acid (ipAA), an algogenic agent considered as "active" stimulus using Duncker's terminology (5), can decrease behavioural responses of animals to nociceptive stimuli, considered as "passive" stimuli. Acetic acid was able to markedly increase threshold for vocalization induced by electrical stimulation of the tail and hot-plate latencies, but tail-flick latencies only in a transient low magnitude manner (4). Kraus *et al.* (6) have proposed that this hypoalgesia triggered by an heterotopic nociceptive stimulus might partially involve neural inhibitory bulbospinal pathways, i.e., serotonin (5-HT) pathways originating from the posterior raphé nuclei [see reference in (1)]. The present study was thus aimed at evaluation of the effects of pretreatment with parachlorophenylalanine (pCPA), a potent 5-HT depletor, upon hypoalgesia induced by ipAA as evaluated with the above-mentioned tests (4). If indeed 5-HT systems are involved in counter-irritation phenomena, then it would be expected that pCPA pretreatment would strongly decrease or suppress the hypoalgesic effects of an heterotopic noxious peripheral stimulus such as the ipAA.

METHOD

One hundred and ninety-two male Sprague-Dawley rats weighing 200–250 g were used. They were housed 5 per cage under diurnal lighting conditions with light on from 08.00 to 20.00 hr and were given food and water ad lib. Behavioural experiments were carried out in a quiet and indirectly lit room, and were performed between 09.00 and 13.00 hr.

For depletion of serotonin, the animals were pretreated with p-chlorophenylalanine, 300 mg/2 ml/kg IP, one injection being given daily for 3 days with the testing session taking place on the

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fourth, while control animals were daily injected with an equivalent volume of saline. This pretreatment results in undetectable levels of spinal 5-HT content (9).

In all experiments, the acetic acid solution was prepared by dissolving 1 g arabic gum in 9 ml of a 1% aqueous solution of acetic acid (pH = 3.20 at 22°C), and vehicle by dissolving 1 g arabic gum in 9 ml of distilled water (pH = 4.31 at 22°C). Each animal received 5 ml/kg IP of one of these solutions.

To assess the effect of each of pCPA pretreatment on animal response to the "active" stimulus, i.e., ipAA injection, a preliminary experiment was performed using the writhing test. Following a 30-min resting period in an individual observing chamber, writhing behaviour was induced in pCPA-pretreated animals (n = 10) by an ipAA injection and the number of writhings was counted per 15-min period during the following 60 min. Control animals (n = 10) were pretreated with the same volumes of saline.

To evaluate counter-irritation effects, three behavioural tests were used as "passive" stimuli: tail-flick test (n = 48), vocalization test (n = 48) and hot-plate test (n = 76), as previously described (4). In all these experiments four groups of animals were determined to allow statistical comparisons: pCPA- or saline-pretreated groups with in each case half of the animals receiving ipAA or IP vehicle solutions injections.

RESULTS

Animal responses to the "active" stimulus (writhing behaviour induced by ipAA injection) were not affected by pCPA pretreatment since in the preliminary experiment the total number of writhes during the 60-min observation period was not significantly affected (14.3 ± 1.1 versus 10.7 ± 2.1 in saline- and pCPA-pretreated rats, respectively).

In two tests, baseline values prior to ipAA injection were significantly increased by pCPA pretreatment: in the tail-flick test, latencies were 2.4 ± 0.2 versus 3.6 ± 0.4 sec in saline- and pCPA-pretreated rats, respectively ($p<0.01$; Student's unpaired t-test); in the vocalization test, thresholds were 1.7 ± 0.1 versus 2.1 ± 0.2 mA in saline- and pCPA-pretreated rats, respectively ($p<0.05$; Student's unpaired t-test). In the hot-plate test, the pCPA-pretreated group displayed no significant differences in jump latencies with the control group.

Effects of pCPA Pretreatment on Counter-Irritation

Tail-flick test (Fig. 1A). In both control and pCPA-pretreated groups, the ipAA injection induced variable, but transitory changes in tail-flick latencies, which in all cases were nonsignificant and of low magnitude.

Vocalization test (Fig. 1B). In both control and pCPA-pretreated groups, the ipAA injection induced a significant increase in the threshold for vocalization, reaching a maximum at 15 min postinjection with a progressive return to baseline level within one hour. While an earlier return to baseline was observed in the pCPA group, its time course did not differ significantly from saline controls (mean surfaces under the curves: 1.74 ± 0.41 and 1.50 ± 0.55 in saline- and pCPA-pretreated groups, respectively).

Hot-plate test (Fig. 1C). In this case, rats were tested only once, and, therefore, comparison has been made between rats receiving vehicle or acetic acid, pCPA pretreated or not, 15 min after IP injection. Jump latency was increased in both groups (pCPA pretreated and control) receiving an ipAA injection, but there was no significant difference between these two groups. In addition, with the analysis of variance test (2×2 factorial paradigm determining the effects of ipAA and/or pCPA pretreatment) using changes in jump latencies values 15 min after ipAA injection, the results show that while acetic acid has a significant effect ($F = 7.31$, $p<0.01$), neither pCPA ($F = 0.18$), nor the interaction of the two factors ($F = 1.05$) is significant.

DISCUSSION

The present results demonstrate that 5-HT depletion induced by pCPA pretreatment is not able to impair hypoalgesia induced by a nociceptive stimulation, a phenomenon known as counter-irritation [see references in (8)].

Behavioural signs induced by various manipulations of 5-HT systems need to be interpreted with caution and more precisely when using pCPA: among other symptoms, pCPA-pretreated rats are overactive, insomniac, hyperglycaemic and react excessively to environmental stimuli [see references in (7)].

This variety of pCPA actions is fairly illustrated by the complexity of the results in the literature concerning its effects on sensitivity to nociceptive stimuli: since the paper of Tenen (12) who first reported an increased sensitivity to high-intensity electrical stimuli in pCPA-pretreated rats, there are more than 30 references in which various tests for nociceptive reactions have been used in pCPA-pretreated rats. The absence of effects of pCPA pretreatment is predominately reported, with the exception of some papers observing an increased sensitivity to a noxious stimulation [see references in (7)]. A decreased reactivity in the tail-flick and vocalization tests have been noted in this work, but no effect in the hot-plate test (see the Results section), data which are in good agreement with others previously described [see references in (7)].

In this study, ipAA injection induced writhing responses in pCPA-pretreated rats. But the failure of pCPA pretreatment to affect the hypoalgesic effect of ipAA is contradictory to pCPA pretreatment effects on other procedures known to induce hypoalgesic effects [see references in (7)]. In the same way, Kraus *et al.* (6) have shown that a 5-HT precursor potentiates the hypoalgesic effect of an IP injected algogen, an effect suppressed by the 5-HT receptor blocker cinanserin. Contradictory results have also been reported when considering the effects of pCPA on hypoalgesia induced by various stressors: increased, unchanged or decreased hypoalgesic effects of stressors by pCPA have been described [see references in (7)]. The large discrepancies encountered in pCPA pretreatment studies concerning hypoalgesic effects of various procedures could be of many origins and it is difficult to propose a rational explanation.

In previous studies from our group (4,8), it has been proposed as a neuronal basis for hypoalgesia resulting from counter-irritation that the activation by nociceptive stimulation of descending inhibitory 5-HT pathways may modulate afferent input in the cord as supported by much data (7). The results reported in this work appear to differ from the implications of this hypothesis since it is shown that the heterotopic hypoalgesic effects of a visceral pain are not affected under experimental conditions that yield undetectable spinal 5-HT levels (9). These results need further confirmation since the effect of pCPA is not totally restricted to 5-HT depletion. In fact, while pCPA has been used as a standard tool to study the physiological role of 5-HT systems, more precise procedures have to be tested such as discrete central nervous system lesions. In this way, in a forthcoming paper it is described that unilateral or bilateral lesions of dorsolateral funiculus, in which descend 5-HT inhibitory bulbospinal pathways [see references in (1)], does not seem to affect hypoalgesia induced by counter-irritation in other counter-irritation paradigms (3).

Counter-irritation is a global process able to diminish inflammation and/or pain restricted to a body area by generating a second

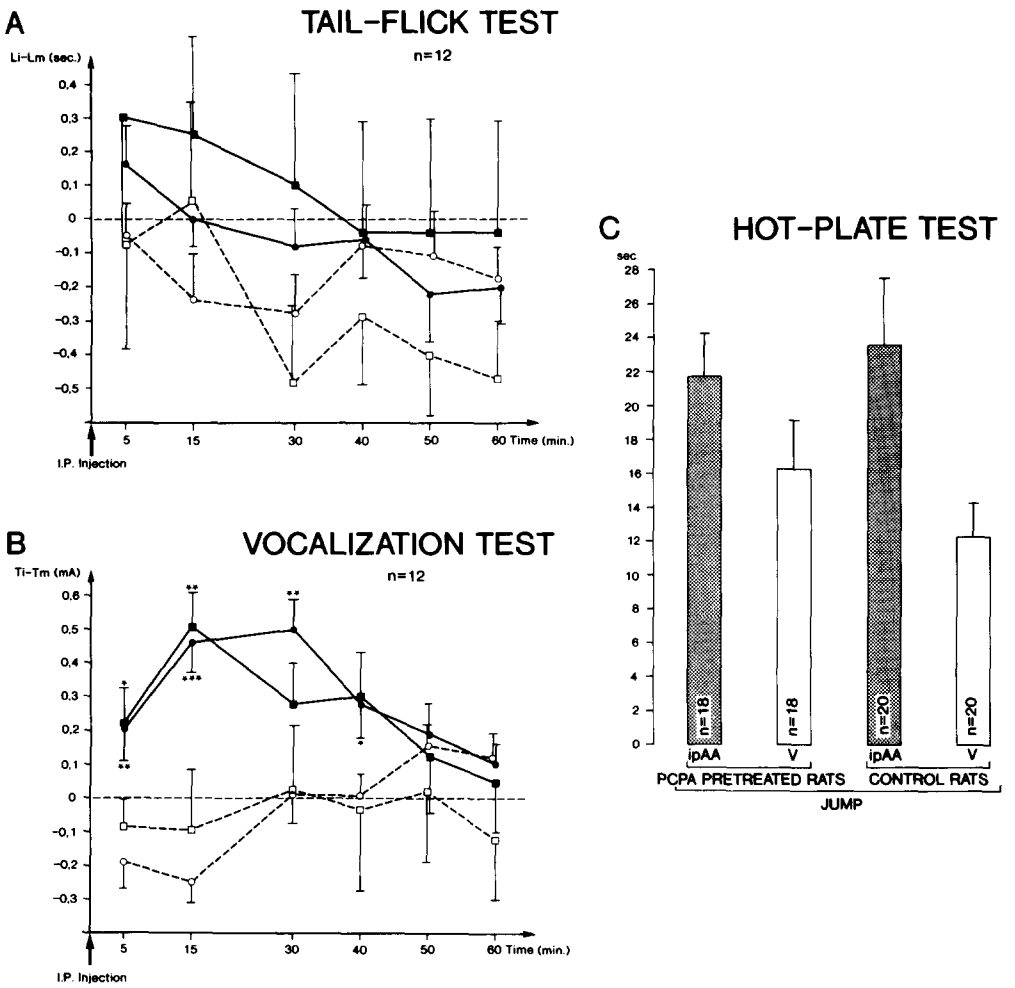

FIG. 1. Time courses of the hypoalgesic effects of IP acetic acid in (A) tail-flick test and (B) vocalization test (see text). In both cases, results are expressed in ordinates as differences between the experimental values at each considered postinjection time and the mean preinjection baselines values (Li-Lm, latencies in sec in A, and Ti-Tm, threshold intensities in mA in B; n = number of animals in each group). Black square symbols, solid line: pCPA-pretreated animals receiving IP acetic acid (ipAA) injection. Open square symbols, dotted line: pCPA-pretreated animals receiving IP vehicle (V) injection. Black circles, solid lines: saline-pretreated controls receiving ipAA. Open circles, dotted lines: saline-pretreated controls receiving IP vehicle. For each considered postinjection time, all data were subjected to the Student's unpaired t-test; when p-values were higher than 0.05, differences were not considered to be significant: (1) ipAA- vs. V-injected animals in saline- and pCPA-pretreated groups, respectively: $*p<0.05$; $**p<0.01$; $***p<0.001$; (2) pCPA- vs. saline-pretreated animals in ipAA- and V-injected groups, respectively: no significant differences. C: Hot-plate test (60 ± 0.5°C). Jump latency (ordinate in sec) was measured in different groups of animals 15 min after IP vehicle (V; open columns) or IP acetic acid (ipAA; dotted columns) injections. This postinjection time was chosen because in a previous study (4) it has been determined that the counter-irritation effect peaked at 15 min, but was no longer present at 30 min. Note the increase in jump latency in both groups receiving an ipAA injection (pCPA-pretreated and control animals). Analysis of variance showed that while acetic acid has a significant effect ($F = 7.31$, $p<0.01$), neither pCPA ($F = 0.18$) nor the interaction of the two factors ($F = 1.05$) is significant (see text).

inflammatory and/or painful focus on a remote body area [see references in (2)]. In that sense it could be possible to consider the involvement of systemic factors different from neurophysiological ones (i.e., 5-HT systems in this study) such as neuroendocrinological factors. The pituitary-adrenal cortical axis or the adrenal medulla could take a part in the mediation of these hypoalgesic effects, since they are mainly involved in the hypoalgesic effect of other procedures close to counter-irritation such as inescapable tail shock or stress [see references in (10,11)]. The possible involvement of such neuroendocrinological factors in our counter-irritation paradigm could explain, at least partly, the failure of pCPA to alter hypoalgesia of counter-irritation origin.

In conclusion, these results tend to reduce the emphasized role of 5-HT systems in counter-irritation (6), and it is possible to suggest that additional mechanisms such as neuroendocrinological factors should also be considered.

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