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Sedative action of chlorinated derivatives of cinnamic acid in the mouse

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We have reported that ferulic acid, *trans*-4-hydroxy-3-methoxycinnamic acid, inhibits the weight gain of rat ventral prostate without any significant effect on seminal vesicles (Saito et al 1979). In the course of our studies on its pharmacological properties, we found that the other derivatives of cinnamic acid, such as 3,4-dichlorocinnamic acid and *p*-chlorocinnamic acid, had sedative and hypnotic actions in the mouse. Analgesic and anti-inflammatory effects of isoferulic acid were also reported (Shibata et al 1975). The present investigation was undertaken to determine quantitatively the inhibitory effects of chlorinated cinnamic acids on the locomotor activity by comparing the effects of some other cinnamic acid derivatives.

Cinnamic acid derivatives were obtained from either Nakarai Chemicals, Kyoto, or Aldrich Chemical Co., Milwaukee, and were predominantly *trans* isomer. Test compounds were dissolved in 0·15 M NaOH, and the solution was neutralized with HCl, then diluted to suitable concentrations with 0·9% NaCl (saline). Female JCL–ICR mice (CLEA Japan, Inc., Tokyo) weighing 25–35 g were maintained on a MF diet (Oriental Manuf. Co.) and free access to water and allowed for at least 7 days to become accustomed to their surroundings before use.

Experiments were carried out in a quiet, well-lighted laboratory area at a room temperature of 25 ± 2 °C. Sedative effects were evaluated by measuring the inhibition of spontaneous locomotor activity with an Animex (model S, LKB Farad). Test compounds were given i.p. immediately before time zero when 3 mice per each group were introduced into the recording cage (37 × 24 × 13 cm transparent plastic) containing a layer of bedding material. Counts of activity were recorded in 5 min blocks for 60 min. Responses during the 20 min period beginning 5 min after drug administration were summed for the calculation of ED50 representing a dose which inhibits the activity by 50% of the control.

Analgesic action was assessed by the hot-plate method (Eddy et al 1950). Plate temperature was 55 °C, and jumping or licking of the paws was used as an endpoint. Test drugs were given s.c. 10, 20, and 30 min before the examination. Acetic acid-induced stretching (Koster et al 1959) was also employed. Mice were given test compounds s.c. and 15 min later 0·6% (v/v) acetic acid was given i.p. in saline (10 ml kg^{-1}). Stretching was counted in a 5 min period starting 10 min after the injection of the acid, and used for the calculation of ED50. Anticonvulsive activity was estimated by the inhibition of tonic convulsion induced either by the injection of strychnine sulphate s.c. (1·5 mg kg^{-1}) or intercorneal application of electroshock (30 mA, 0·2 s). Acute toxicity was determined by the fatalities at 72 h later with a single injection of test compounds. All ED50 and LD50 values and their 95% confidence limits were calculated by probit analysis (Finney 1971).

When the mouse was given i.p. *p*-chlorocinnamic acid, 90 mg kg^{-1}, the acute symptoms observed were a clear decrease in spontaneous motility without loss of body posture and responsiveness to external stimuli. The inhibitory effects on the motility were measured by counting activities of groups of 3 mice with the Animex. When introduced into an unfamiliar cage, saline-treated control mice showed a consistent and sustained locomotor activity for about 20 min (Fig. 1). This activity consists primarily of exploratory behaviour and gradually decreases to a low level as the surroundings become familiar. Intraperitoneal administration of chlorinated cinnamic acids immediately before trans-

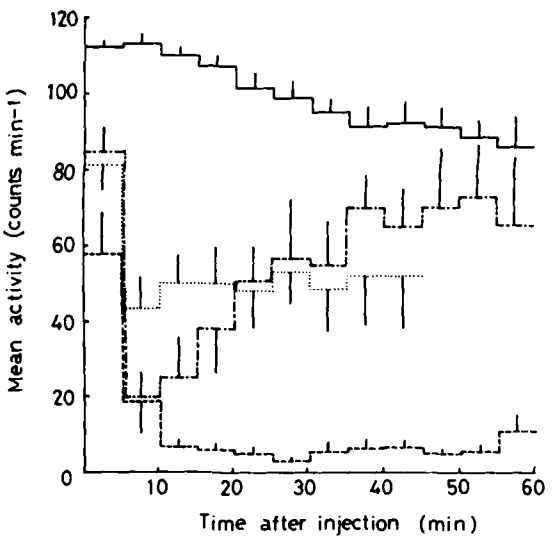

FIG. 1. Time course for the inhibition of spontaneous locomotor activity by chlorinated cinnamic acids. Drugs (90 mg kg^{-1}) or saline were given i.p. at time zero to the groups of 3 mice. The number of groups (n) is as follows: (——, n = 9), saline; (-----, n = 6), 3,4-dichlorocinnamic acid; (–·–·–, n = 5), *p*-chlorocinnamic acid; (·····, n = 5), *o*-chlorocinnamic acid. Vertical bars indicate the s.e.

* Correspondence.

location into the recording cage produced the transient or persistent suppression of the exploratory response (Fig. 1). By measuring the activity counts in a period from 5 to 25 min after drug administration, the inhibition of exploratory activity could be quantified and its intensity is shown with respect to the position and the kinds of substituents in cinnamic acid (Fig. 2). The potent compounds were further examined at different doses and ED50s (with confidence limits) are as follows: 3,4-dichlorocinnamic acid, 52 (38–67) mg kg^{-1}; *p*-chlorocinnamic acid, 78 (64–97) mg kg^{-1}; *m*-chlorocinnamic acid, 85 (61–119) mg kg^{-1}; *o*-chlorocinnamic acid, 87 (55–128) mg kg^{-1}. All of the chlorinated cinnamic acids tested had steep dose-response curves: twofold doses of these compounds covered the range of activity from nearly 0 to 100% inhibition.

One minute after i.p. injection of 100 mg kg^{-1} of 3,4-dichlorocinnamic acid, mice did not move spontaneously but walked with ataxic gait responding to stimuli. The hind limbs were dragged. Corneal reflex, righting reflex, and stretch reflexes of the abdominal wall and limbs were positive. Five minutes after the injection mice could hold on themselves to a horizontal rotating wooden bar. At the dose of 200 mg kg^{-1}, the animals showed ataxic gait and could not hold on to the bar. The righting reflex was lost after 5 min. However, the stretch reflexes were even stronger than with the smaller doses. No sign of respiratory depression was seen. These findings suggest that the drugs are acting centrally.

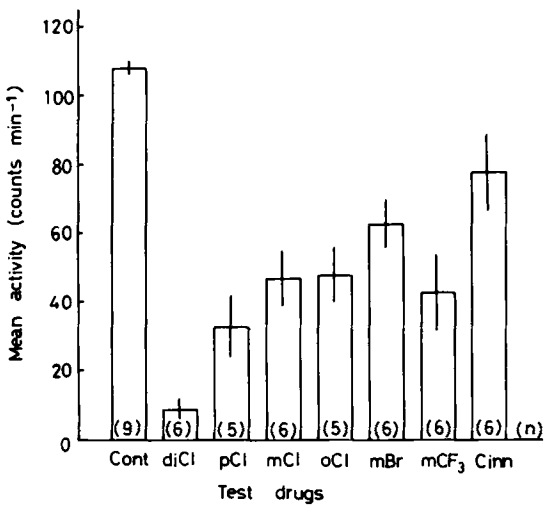

FIG. 2. Inhibition of spontaneous locomotor activity by cinnamic acid derivatives. Activity counts generated during the 20-min block started 5 min after drug administration (90 mg kg^{-1}, i.p.) were summed for comparison. Each column represents the mean of trials (n) performed 3 mice per group. Vertical bars indicate the s.e. Cont, control (saline); diCl, 3,4-dichlorocinnamic acid; pCl, *p*-chlorocinnamic acid; mCl, *m*-chlorocinnamic acid; oCl, *o*-chlorocinnamic acid; mBr, *m*-bromocinnamic acid; mCF_3, *m*-(trifluoromethyl)-cinnamic acid; Cinn, cinnamic acid.

The analgesic action of 3,4-dichlorocinnamic acid was observed in the acetic acid-induced stretching method; the ED50 was 51 (34–87) mg kg^{-1}, s.c. However, this drug did not prolong the reaction time in the hot-plate method at doses up to 100 mg kg^{-1}, s.c. The i.p. route was not suitable for the measurement of analgesic activity, as the duration of the effect was so short that the writhing reactions were not produced in time, and acetic acid, which had to be injected i.p., might have precipitated the drugs owing to low pH. The drugs, therefore, were given s.c. in the analgesic tests. Anticonvulsive effect of 3,4-dichlorocinnamic acid was not detected either in the present methods up to 100 mg kg^{-1}, i.p. At doses above 100 mg kg^{-1}, however, this compound significantly delayed the strychnine-induced death.

The LD50 values of 3,4-dichlorocinnamic acid were 267 (227–323) mg kg^{-1}, i.p. and 442 mg kg^{-1}, s.c., and that of *p*-chlorocinnamic acid was 590 (444–871) mg kg^{-1}, i.p. Toxicity of these compounds was characterized by ataxia and loss of righting reflex with lower doses, and severe respiratory suppression and clonic convulsion ending in death with higher doses.

As a conclusion, cinnamic acid derivatives chlorinated in the aromatic ring have sedative-hypnotic activity. Among those studied so far, 3,4-dichlorocinnamic acid showed the most potent inhibition of spontaneous motility, although the toxicity appeared to be the greatest. The inhibition was also observed at higher doses of *p*-coumaric acid and caffeic acid, but not of cinnamic acid and ferulic acid (data not shown). Therefore, the inhibitory potency of the substituents in the aromatic ring of cinnamic acid in descending order is as follows: Cl, $CF_3 \geqq Br > OH \gg H$, OCH_3. The potency seems to be influenced not by the position of chloride in aromatic ring, but by the number of the substituents. Structurally, *p*-chlorocinnamic acid is thought as analogous to phenaglycodol with respect to its sedative effect. The apparent analgesic action of 3,4-dichlorocinnamic acid observed in the stretching method may partly be due to the immobility of the animals brought about by the drug.

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