Antagonism of Picrotoxin Against the Taming Effect of Carbamazepine on Footshock Induced Fighting Behavior in Mice

Kenzo NAKAO, Takahiro HIGASHIO and Toshiya INUKAI

Biological Research, Research and Development Department, Pharmaceutical Division, Ciba-Geigy Japan, 10-66 Miyuki-cho, Takarazuka, Hyogo 665, Japan

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Abstract—Carbamazepine (10 and 20 mg/kg, i.p., P<0.01; 40 mg/kg p.o., P<0.01), Li2CO3 (200 mg/kg, i.p., P<0.01; 200 mg/kg, p.o., P<0.05), diazepam (0.5 mg/kg, i.p., P<0.01) and haloperidol (0.5 mg/kg, i.p., P<0.01) significantly decreased the number of fighting episodes induced by footshock in mice. Picrotoxin (0.3 mg/kg, s.c.) and bicuculline (0.5 mg/kg, s.c.) antagonized the effect of carbamazepine or diazepam completely. Our present results suggest that the taming property of carbamazepine in the footshock model have some relation with the GABAergic mechanism.

The anti-manic effect of carbamazepine was reported by Takezaki and Hanaoka (1) and by Okuma et al. (2). Carbamazepine shows anti-convulsive action, antagonism against methamphetamine and inhibits the conditioned avoidance response in animals (3). Carbamazepine also decreases the fighting behaviors in isolated mice (4). Concerning the mechanism of the anti-convulsive action of carbamazepine, its relation to GABA, an inhibitory transmitter in the CNS, does not seem to have much importance (5). However, it is not clear whether GABA transmission is attributable to the taming effect of carbamazepine or not. Footshock induced aggressive behaviors seem to be one of the suitable screening methods for human hyperirritability (6). In the present study, we examined the effect of carbamazepine in the single administration or in the combination with picrotoxin, a GABA-regulated chloride ion channel blocker (7), bicuculline, or with pentetrazole, another GABAergic antagonist (8) in this model.

Male ddY mice, 5–6 weeks of age, weighing about 25 g, were purchased from Shimizu Experimental Materials Co., Ltd. According to the methods of Tedeschi et al. (9) and Ueki et al. (10), pairs of mice were confined in an acrylic-fiber cylinder (17.5 × 25 cm) with a steel grid floor and were subjected to electric shocks (5 Hz, 0.2 msec, 150 V, 3 min). Each succession of fighting behavior, with both mice standing on their hind legs, sparring and biting at each other was counted as one fighting episode. Only those pairs which exhibited more than four episodes within 3 min of the control schedule were used for further experiments. The fighting episodes were again recorded at 60 min after the drug administration. Drugs used were as follows: carbamazepine (Ciba-Geigy), p.o. and i.p., suspended in 0.5% carboxymethyl cellulose sodium salt solution (CMC); Li2CO3 (Kanto Chemicals), p.o. and i.p., dissolved in distilled water and saline respectively; diazepam (Koel Chemicals), i.p., dissolved in 0.5% CMC; haloperidol (Dainippon, serenase inj.), i.p., dissolved in saline; picrotoxin (Sigma) and pentetrazole (Sigma) dissolved in saline. Bicuculline (Sigma) was dissolved in a small volume of 0.1 N HCl and diluted with saline. Drugs used were given at the volume of 10 ml/kg b.w. Picrotoxin, bicuculline or pentetrazole was subcutaneously challenged at 50 min after the carbamazepine, Li2CO3, diazepam or haloperidol administration (at 10 min before the 2nd footshock). For the statistic analysis, Student’s paired t-test was used.

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(200 mg/kg, i.p., P<0.01; 200 mg/kg, p.o., P<0.05), diazepam (0.5 mg/kg, i.p., P<0.01) and haloperidol (0.5 mg/kg, i.p., P<0.01) significantly decreased the number of fighting episodes in mice (Table 1).

Picrotoxin (0.3 mg/kg, s.c.) antagonized the effect of carbamazepine (10 mg/kg, i.p.) or diazepam (0.5 mg/kg, i.p.) completely. Picrotoxin (0.3 mg/kg, s.c.) did not, however, inhibit the effect of Li$_2$CO$_3$ or haloperidol on the fighting episodes. Bicuculline (0.5 mg/kg, s.c.) antagonized the effect of carbamazepine or diazepam. Pentetrazole (20 mg/kg, s.c.) also antagonized the effect of carbamazepine. Picrotoxin (0.1 and 0.3 mg/kg, s.c.), bicuculline (0.1 and 0.5 mg/kg, s.c.) and pentetrazole (20 mg/kg, s.c.) showed no effects when used alone in this model (Table 2).

Carbamazepine even at a dose of 20 mg/kg, i.p., showed little analgesic effect (3), so the analgesic effect seemed not to contribute to the results.

Benzodiazepines are considered to act, at least in part, by enhancing GABAergic synaptic transmission (7). Antagonism of the taming effect of diazepam by GABA antagonists found in the present study may support the above hypothesis.

Since carbamazepine is known to have some affinity for the [³H]-diazepam binding site (11), it is probable that carbamazepine shares a common action mechanism with diazepam. In fact like diazepam, the taming effect of carbamazepine was antagonized by GABA antagonists, picrotoxin, bicuculline or pentetrazole.

These results may suggest that part of the taming effect of carbamazepine is also associated with GABA transmission, although it does not exclude the possibility that the antagonism was partly brought about by non-GABAergic secondary actions.

Table 1. Effects of carbamazepine, Li$_2$CO$_3$, diazepam and haloperidol on footshock induced fighting episodes in mice

| Drugs          | N  | Dose (mg/kg) | Fighting counts (mean±S.E.) before treatment (control) | after treatment |
|---------------|----|--------------|-------------------------------------------------------|-----------------|
| Saline        | 12 | —            | 9.3±0.9                                               | 10.1±1.3        |
| Carbamazepine | 9  | 10 (i.p.)    | 10.2±1.3                                              | 5.9±1.2**       |
|               | 10 | 20 (i.p.)    | 12.1±1.7                                              | 3.0±0.9**       |
|               | 8  | 20 (p.o.)    | 12.0±2.7                                              | 9.5±2.0         |
|               | 9  | 40 (p.o.)    | 9.7±1.0                                               | 4.1±1.4**       |
| Li$_2$CO$_3$  | 8  | 100 (i.p.)   | 9.8±1.0                                               | 8.3±1.7         |
|               | 8  | 200 (i.p.)   | 13.5±1.9                                              | 6.6±1.4**       |
|               | 8  | 100 (p.o.)   | 10.3±1.7                                              | 9.9±1.5         |
|               | 8  | 200 (p.o.)   | 11.1±1.8                                              | 8.3±1.2         |
| Diazepam      | 10 | 0.5 (i.p.)   | 11.7±1.5                                              | 4.2±2.0**       |
| Haloperidol   | 15 | 0.5 (i.p.)   | 9.4±0.9                                               | 1.1±0.4**       |

*P<0.05, **P<0.01: significantly different from before treatment (control).  N: number of pairs.

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| Drugs (Dose)               | N | Fighting counts (mean±S.E.) before treatment (control) | after treatment |
|---------------------------|---|--------------------------------------------------------|-----------------|
| Picrotoxin                | 8 | 7.3±0.9                                                | 7.3±1.0         |
| (0.1 mg/kg, s.c.)         | 8 | 7.8±0.7                                                | 5.4±0.9         |
| Carbamazepine             | 8 | 10.0±0.6                                               | 6.4±1.1**       |
| (10 mg/kg, i.p.)          | 8 | 8.5±1.1                                                | 7.4±1.1         |
| + Picrotoxin              | 8 | 9.3±1.3                                                | 5.9±1.6*        |
| (0.1 mg/kg, s.c.)         | 8 | 8.1±1.0                                                | 6.0±1.4         |
| Li2CO3                    | 8 | 7.4±0.8                                                | 1.4±0.8**       |
| (200 mg/kg, i.p.)         | 8 | 8.9±1.5                                                | 8.8±1.7         |
| + Picrotoxin              | 8 | 8.6±1.2                                                | 8.1±1.2         |
| (0.3 mg/kg, s.c.)         | 8 | 11.0±1.9                                               | 6.9±1.2**       |
| Bicuculline               | 8 | 9.0±1.2                                                | 8.8±1.2         |
| (0.1 mg/kg, s.c.)         | 8 | 10.0±1.3                                               | 9.6±1.5         |
| Carbamazepine             | 8 | 11.3±1.2                                               | 11.6±1.7        |
| (10 mg/kg, i.p.)          | 8 | 12.4±1.4                                               | 10.9±2.2        |
| + Bicuculline             | 8 | 10.5±1.0                                               | 8.9±1.2         |
| (0.5 mg/kg, s.c.)         | 8 | 9.5±1.2                                                | 7.8±1.6         |
| Diazepam                  | 8 | 10.0±1.3                                               | 9.6±1.5         |
| (0.5 mg/kg, i.p.)         | 8 | 11.3±1.2                                               | 11.6±1.7        |
| Pentetrazole (20 mg/kg, s.c.) | 8 | 12.4±1.4                                               | 10.9±2.2        |

*P<0.05, **P<0.01: significantly different from before treatment (control). N: number of pairs. Picrotoxin, bicuculline or pentetrazole was challenged at 10 min before the 2nd footshock. The 2nd footshock was given at 60 min after carbamazepine, Li2CO3, diazepam or haloperidol administration.