Facilitating Effect of Oxiracetam and Piracetam on Acquisition of Discrete Two-Way Shuttle Avoidance in Normal Mice

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Abstract—Effects of oxiracetam and piracetam on acquisition of the discrete two-way shuttle avoidance response were investigated in normal mice of the dd strain. When oxiracetam or piracetam was administered only once immediately before the training session, the mice showed a greater number of avoidance responses in comparison with the saline-treated control mice, with the maximum effect at 30 mg/kg of oxiracetam and 100 mg/kg of piracetam. These results suggest that oxiracetam and piracetam facilitate the avoidance acquisition in normal mice.

Piracetam, a cyclic GABA derivative, is thought to activate brain integrative mechanisms without eliciting behavioral excitation (1). Piracetam was followed by many analogous compounds such as aniracetam (2) and oxiracetam (3). It has been reported that these compounds enhance learning and/or memory in brain-damaged animals (1–5), in aged animals (6), and even in normal animals (7, 8) in one-way active avoidance, passive avoidance and/or maze situations. Recently, Sansone et al. (9) studied effects of piracetam and oxiracetam on the acquisition of discrete two-way shuttle avoidance in two strains (BALB/c and C57BL/6) of inbred mice. They reported a marked facilitation of the avoidance acquisition in BALB/c mice when the training (5-daily sessions of 100 trials each) was preceded by the 5-day pretreatment regimen either piracetam (100 mg/kg, i.p.) or oxiracetam (50 mg/kg, i.p.), but not by a 5-day treatment regimen with the same drug doses when they were injected 30 min before the start of each training session. Therefore, Sansone et al. suggested that a pretreatment for several days was a minimum requirement to observe the facilitating-effect of piracetam and oxiracetam on the acquisition of discrete two-way shuttle avoidance in normal mice.

The purpose of this experiment was to study whether single administration of oxiracetam and piracetam was effective for facilitating acquisition of the discrete two-way shuttle avoidance in mice.

The experimental animals were male mice of the dd strain (Institute of Experimental Animal Research, Gunma University School of Medicine). We (10) have shown that this mouse strain rapidly acquires a discrete two-way shuttle avoidance and that the acquisition process is reliable and reproducible. When these mice were 8 weeks of age and weighed 28–32 g, the training of discrete two-way shuttle avoidance was started.

Oxiracetam and piracetam were dissolved in physiological saline immediately before use. The doses tested were 10, 30 and 100 mg/kg, i.p., for both of the drugs, and each injection volume was always constant at 0.1/10 g body weight.

Five equivalent shuttle boxes (GT-8450, O'Hara & Co., Ltd.) were used as described previously (10–12). Briefly, the shuttle box was 30(W)×9(D)×15(H) cm, to which 2 infrared photo-beams, arranged 18 cm apart, were attached. A speaker for presenting a warning stimulus was located in the center ceiling of the box. Solid state equipment (De CARES GT-M5; O'Hara & Co., Ltd.) simultaneously controlled and recorded the behaviors of each of 5 mice. The shuttle boxes were placed in sound-attenuating chambers.

The temporal parameters of the discrete avoidance schedule (13) consisted of an...
intertrial interval of 22 sec and a warning duration of 5 sec. The warning stimulus was an 800 Hz tone. An electric shock of 150 V, 0.5 mA, 50 Hz AC was given to the mouse through a stainless steel floor grid of the shuttle box. The maximum duration of the shock presentation was 3 sec, but an escape contingency was inserted in the schedule. Thus, an avoidance response was recorded when the mouse avoided the shock by running in the box and cutting the photo-beam at the opposite side within 5 sec after the onset of the warning stimulus. Each training session consisted of 1-hr training per day, during which 120 avoidance trials were carried out at intervals of 30 sec. In this experiment, 2 training sessions were held at a 24 hr interval. Drugs or physiological saline (control injection) were administered only once immediately before the start of the 1st session. Twenty animals were used in each drug-testing, and 2 groups of 20 animals received saline as the control. The experiment was conducted between 9 a.m.–3 p.m.

The avoidance acquisition progressively succeeded in each training session. Therefore, numbers of the avoidance responses were calculated every 20 avoidance trials to observe the acquisition process according to our previous study (12).

At first, the overall data were analyzed by a repeated measures analysis of variance (ANOVA). If there were significant overall effects, comparisons between the individual mean values were conducted by the two tail Student’s t-test. When P values were equal to or less than 0.05, they were considered to be significantly different.

One mouse in the saline-treated control group was accidentally killed by passage of electric current through the brain when it bit the floor grid. Since the saline-treated groups

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**Fig. 1.** Effects of oxiracetam (10, 30 and 100 mg/kg, i.p.) on acquisition of the discrete two-way shuttle avoidance in mice. The drug or physiological saline (control injection) was administered only once immediately before the start of the 1st training session. The 2nd training session was held 24 hr after the 1st session without any additional treatment. Each training session lasted for 1 hr, during which 120 avoidance trials were carried out at intervals of 30 sec. Each point indicates the mean avoidance responses during every 20 avoidance trials. *indicates a significant difference from the saline-treated control value (P<0.05).
demonstrated similar results, these data were combined. Thus, the number of control mice was 39.

Figures 1 and 2 show the effects of oxiracetam and piracetam, respectively, on the acquisition of the discrete two-way shuttle avoidance in the mouse. In these figures, the mean numbers of the avoidance responses during the block of every 20 avoidance trials are presented. Since the number of shuttles were 21–28/block, and there was no significant difference between the saline-treated and drug-treated groups, these data were not presented in the figure. The analysis of variance of the overall avoidance responses revealed that there were significant drug effects and trial-by-treatment interactions. The results of individual comparisons are presented in each figure. Briefly, both oxiracetam and piracetam facilitated the avoidance acquisition not only in the 1st session but also in the 2nd session. The maximum effects were observed at 30 mg/kg of oxiracetam and 100 mg/kg of piracetam.

A gross observation revealed that neither sedation nor excitation was detectable in the mice after injection of these drugs.

The present experiment demonstrated that the acute administration of either oxiracetam or piracetam facilitated acquisition of a discrete two-way shuttle avoidance in normal mice of the dd strain. Our results are comparable with those reported by Sansone et al. (9) that 5-daily, but not acute, pretreatment with oxiracetam (50 mg/kg) or piracetam (100 mg/kg) was effective for facilitating the acquisition of two-way shuttle avoidance in mice in spite of differences in the experimental conditions from ours that included mouse strain, shuttle box, warning signal, drug administration regimen.

In the shuttle avoidance situation, an increase in general activity results in a facilitation of the avoidance behavior (10, 14). However, such a non-specific factor is unlikely to explain the present results, since gross observation failed to detect any evidence of a drug-induced behavioral excitation. In addition, the drug doses tested in this experiment elicited a marked change in neither the established discrete two-way shuttle avoidance response nor the ambulatory
(locomotor) activity (H. Kuribara, unpublished data). Furthermore, at higher doses, these drugs tended to suppress the avoidance response and ambulatory activity, and they reduced the ambulation-increasing effect of methamphetamine.

The improvement of learning and memory by piracetam has been ascribed to its effects on interhemispheric communication, integrative mechanisms and memory retrieval process (1, 15). Although piracetam and oxiracetam are cyclic GABA derivatives, these drugs hardly affect GABAergic function, but rather activate cholinergic function in the brain (16, 17). In addition, we (12) have demonstrated that amiridin, an acetylcholinesterase inhibitor, is effective for facilitating the avoidance acquisition of the normal dd mice in the same experimental situation. In these respects, it seems that the facilitation of avoidance acquisition induced by oxiracetam and piracetam is partially mediated through a stimulant action on central cholinergic function.

The dose-effect relation revealed that the effect of oxiracetam shows an inverted U shape with maximum effect at 30 mg/kg. Whereas, piracetam exhibited the maximum effect at 100 mg/kg. According to our experience, and the observations reported by Giurgea and Salama (1) and Schindler et al. (8), the behavioral effect of piracetam was less at 300 mg/kg than at 100 ng/kg. It is therefore considered that dose-effect curve of piracetam also shows an inverted U shape, similar to that of oxiracetam.

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