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BRIEF COMMUNICATION

Prolongation of Latencies for Passive Avoidance Responses in Rats Treated with Aniracetam or Piracetam

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YAMADA, K., T. INOUE, M. TANAKA AND T. FURUKAWA. *Prolongation of latencies for passive avoidance responses in rats treated with aniracetam or piracetam.* PHARMACOL BIOCHEM BEHAV 22(4) 645–648, 1985.—Effects of aniracetam (1-anysoyl-2-pyrrolodone) and piracetam (1-acetamido-2-pyrrolidone) on passive avoidance behavior were studied in 2 and 18 months old rats using a step-down passive avoidance task. Repeated administration of aniracetam (30 and 50 mg/kg, IP × 5 days) or piracetam (100 mg/kg, IP × 5 days) significantly prolonged step-down latencies for a passive avoidance task in 2 months old rats. Administration of aniracetam (50 mg/kg, IP) or piracetam (100 mg/kg, IP), however, did not affect locomotor activity. This prolongation of latencies was also seen with oral administration of aniracetam (50 mg/kg × 5 days). Similar prolongation of latencies also occurred in 18 months old rat treated with aniracetam (50 mg/kg, IP × 5 days). The results imply that aniracetam may improve learning and/or memory in 2 and 18 months old rats.

Aniracetam Piracetam Passive avoidance behavior Locomotor activity

VARIOUS lines of experiments have been done in quest of effective remedies for cognitive decline in man [2, 16–18]. Some peptides and γ-aminobutyric acid (GABA) have been reported to improve cognitive functions although neuronal mechanisms involved in cognitive functions are still far from being understood [4, 6, 10, 15]. Piracetam, 1-acetamido-2-pyrrolidone (Fig. 1), which is a cyclic derivative of GABA, has been reported to enhance learning and memory and to protect these functions against hypoxia in animal studies [7, 8, 13]. Clinical experiments also show that piracetam improved overall functioning, particularly alertness, socialization and cooperation in elderly psychiatric patients with mild diffuse cerebral impairment [2]. Recently, another cyclic derivative of GABA, aniracetam (1-anysoyl-2-pyrrolodone) (Fig. 1) has been selected from a series of related compounds and is expected to have the character of cognitive activator [3,11].

The present study was, therefore, designed to determine whether or not aniracetam would improve the ability to learn or recall in 2 and 18 months old rats.

METHOD

Male Wistar rats (2 and 18 months old rats) were obtained from Kyudo Animal Laboratory (Kumamoto, Japan) and maintained in air conditioned laboratories at a temperature of 23±1°C in a 12 hour light-dark cycle (7:00 a.m.–7:00 p.m.). Commercial food (CE-2, Clea, Ltd., Japan) and tap water were available ad lib except for during the time of the experiments.

A step-down passive avoidance apparatus similar to that previously described [15] was used. In brief, the apparatus consisted of two compartment divided by a guillotine door (15×15 cm) into a small illuminated chamber (15×15×65 cm) and larger darkened chamber (60×60×70 cm). The large darkened chamber was made up of electrifiable grid floor and the shock was delivered to the animal's feet with a shock generator-scrambler (SGS-004, BRS/LVE).

On the adaptation day, rats were allowed to explore the step-down passive avoidance apparatus for 16 min. On the training day, each rat was placed in the small chamber and 60 sec later the door was opened, starting an electric timer. The moment the rat stepped down to the darkened compartment (defined as the passage of the hind legs over the threshold), the door was closed, stopping the timer and foot shock (0.8 mA, for 5 sec) was given three times to the animal's feet. The rat was then removed promptly from the apparatus and placed into its home cage. Twenty-four hours after training, each rat was placed into the small box, the door was opened 60 sec later and step-down latency was measured. This response latency was timed to an arbitary maximum of 900 sec. Drugs and vehicle were given once a day for 5 days. Rats received drugs and vehicle for 2 days before adaptation, and, on the adaptation, training and test days, drugs and vehicle were also given 1 hour before placing rats into the small chamber.

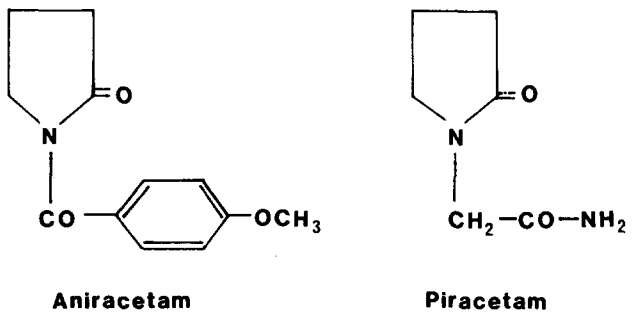

FIG. 1. Chemical structures of aniracetam (Ro 13-5057, 1-anysoyl-2-pyrrolodone) and piracetam (UCB 6215, 1-acetamido-2-pyrrolidone).

For measurement of locomotor activity, each rat was placed in a transparent plastic cage (35×30×17 cm) mounted on the top of an Automex meter (Columbus Instrument Co.). Rats were grouped into 8, thereby balancing the number of counts measured prior to the experiments. Locomotor activity was measured for 5 min at 15, 30, 45 and 60 min after a single administration of drugs. Aniracetam (Ro 13-5057, Roche, Basel, Switzerland) and piracetam (UCB 6215, UCB Nordiska, Stockholm, Sweden) were administered intraperitoneally (IP) or orally (PO) as a suspension in 0.5% carboxymethyl cellulose. Doses of aniracetam (50, 100 mg/kg) and piracetam (100 mg/kg) were selected in the present experiment in accordance with previous animal studies [3, 8, 11, 13]. Control groups were treated with 0.5% carboxymethyl cellulose.

The step-down latency was expressed as median value and statistical analysis was calculated using either the Fisher exact probability test or the Chi square test. Locomotor activity was expressed as mean value and results were evaluated using a one-way analysis of variance followed by the Dunnett's t-test [19]. The level of significance (two-tailed test) chosen was $p<0.05$.

RESULTS

Prolongation of Step-Down Latencies for a Passive Avoidance Task in Youthful Rats Treated With Aniracetam or Piracetam

Figure 2 shows the median step-down latencies during the test trial in 2 months old rats. The non-foot shock groups, which were treated with vehicle or drugs but were not given electrical shock on the training day, immediately got out from a small chamber to a large chamber, the median step-down latencies being less than 30 sec. However, the foot shock groups treated with vehicle had a significantly longer step-down latency in comparison with the non-foot shock group treated with vehicle. Treatment with aniracetam (30, 50 mg/kg, IP × 5) or piracetam (100 mg/kg, IP × 5) for 5 days significantly increased the median step-down latencies as compared with the foot-shock group treated with vehicle. Abnormal behavior including ataxia was not observed in rats treated with these doses of aniracetam or piracetam.

Oral administration of aniracetam (50 mg/kg, PO × 5 days) also significantly increased the step-down latencies, the median latencies being 278 sec in vehicle-treated group (n=15) and 900 sec in aniracetam-treated group (n=15), respectively.

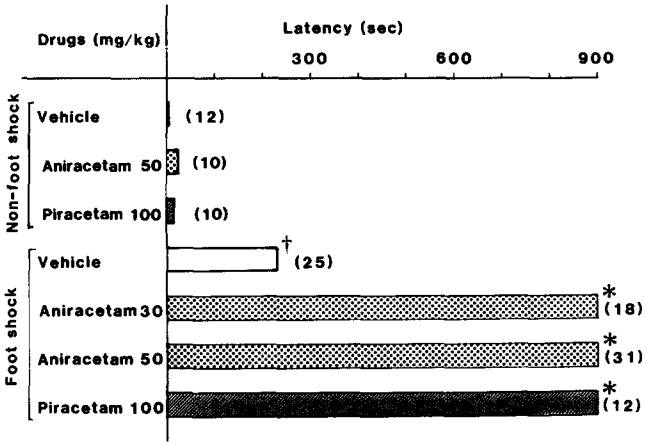

FIG. 2. Effects of aniracetam and piracetam on a step-down passive avoidance responses in youthful 2 months old rats. Rats received aniracetam (30 and 50 mg/kg, IP) piracetam (100 mg/kg, IP) and vehicle (0.5% carboxymethyl cellulose) once a day for 5 days: 2 days before adaptation as well as adaptation, training and test days. Drugs and vehicle were given 1 hour before placing rats into the small chamber. Each horizontal bar represents the median test latency. Numbers in parentheses indicate numbers of rats used. †$p<0.05$, significant difference from the vehicle group which was not given foot shock in training day. *$p<0.05$, significant difference from the vehicle group which was given foot shock.

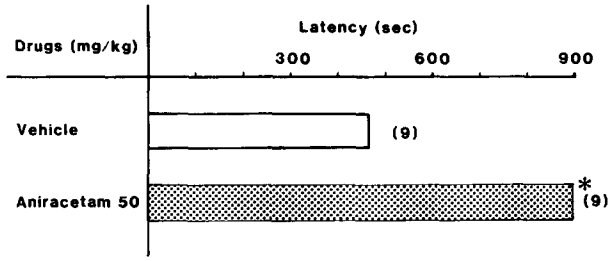

FIG. 3. Effects of aniracetam on a step-down passive avoidance responses in elderly 18 months old rats. Aniracetam (50 mg/kg, IP) was given once a day for 5 days. *$p<0.05$, significant difference from the vehicle group. See Fig. 2 for further details.

Prolongation of Step-Down Latencies for a Passive Avoidance Task in Elderly Rats Treated With Aniracetam

In this experiment, 18 months old rats were used. As shown in Fig. 3, aniracetam (50 mg/kg, IP × 5 days)-treated group had a significantly longer step-down latency (900 sec) than that (465 sec) in vehicle-treated group.

Effects of Aniracetam and Piracetam on Locomotor Activity in Youthful Rats

Table 1 shows locomotor activity after a single administration of aniracetam (50 mg/kg, IP) or piracetam (100 mg/kg, IP) in 2 months old rats. Locomotor activity in vehicle-treated group gradually decreased and treatment with aniracetam or piracetam did not affect locomotor activity in rats.

DISCUSSION

The long-term effects of piracetam has been reported to exert therapeutic effects in a wide variety of disorders [2, 5,

TABLE 1

EFFECTS OF ANIRACETAM AND PIRACETAM ON LOCOMOTOR ACTIVITY IN 2 MONTHS OLD RATS

	Counts/5 min (Mean ± SE)				
Drugs (mg/kg, IP)	0	15	30	45	60 min
Vehicle	61.3 ± 11.0	45.5 ± 9.1	30.6 ± 11.2	12.0 ± 4.0	17.4 ± 4.6
Aniracetam 50	59.0 ± 10.9	44.3 ± 17.5	26.5 ± 6.8	10.5 ± 4.4	15.3 ± 4.5
Piracetam 100	65.0 ± 15.7	39.1 ± 9.1	26.9 ± 7.7	23.9 ± 5.4	26.5 ± 9.0

Each value represents the mean ± 1 S.E. of the counts of locomotor activity during 5 min as measured from 8 rats.

There were no significant difference between vehicle and aniracetam or piracetam-injected groups at any time as determined by a one-way analysis of variance followed by the Dunnett's *t*-test.

12]. Most of animal experiments on cognitive effects with piracetam were also investigated in schedules of multiple drug administration before and after one or more training sessions [8, 20, 21]. Therefore, in these experiments, drugs were administered once a day for 5 days including adaptation, training and test days in a passive avoidance task. Piracetam (100 mg/kg, IP × 5 days) considerably prolonged step-down latencies for a passive avoidance response. This effect is in general agreement with that observed by Wolthuis [20,21] who reported that piracetam and etiracetam, a structural analogue of piracetam, enhanced acquisition on a Y-maze discrimination learning or improved retention on passive avoidance learning in rats.

Repeated administration for 5 days of aniracetam (50 and 100 mg/kg, IP) also prolonged step-down latencies in a passive avoidance task. This prolongation of step-down latencies in the foot-shock group may not be due to motor incapacitation since the treatment with aniracetam did not prolong latencies in the non-foot shock group and failed to affect locomotor activity as measured by an Automex meter. Therefore, aniracetam might improve learning and/or memory. Moreover, the prolongation of step-down latencies was seen with repeated oral administration of aniracetam (50 mg/kg × 5 days). Aniracetam has been reported to prevent short-term amnesia in a passive avoidance situation induced by scopolamine, chloramphenicol, cychoheximide or electroconvulsive shock given immediately after training [3,11]. The improving action of piracetam on cognitive decline has been proposed to be due to its increasing adenosine triphosphate formation [14] and enhancing protein synthesis [1] in cortical nerve cells, the mechanisms underlying the improving action of aniracetam are, however, scarcely known. Recently, Kubota *et al.* [11] found that aniracetam itself did not change glucose utilization rate in many brain areas, but selectively prevented the scopolamine-induced decrease of the cerebral activity (glucose utilization rate) in the two ascending activating systems, the reticulo-thalamo-neocortex system and hypothalamo-archi/paleocortex system which might be involved in memory and learning.

Giurgea and Mouravieff-Lesuisse [9] reported that 12 months old rats crossed a water maze with significantly fewer errors than controls when they were pretreated with piracetam (45 mg/kg, IP) 30 min before the learning session. Giurgea *et al.* [7] also found an enhanced learning ability in rats that were about 9 months old for a 'therehold' active avoidance task if the animals were injected a minimal dose of 10 mg/kg, SC 1 hour before training. Then, we also studied in 18 months old rats the effects of aniracetam on a passive avoidance task. Repeated administration of aniracetam (50 mg/kg, IP × 5 days) prolonged step-down latencies in 18 months old rats. Therefore, it would appear that repeated treatment with aniracetam also improves learning and/or memory in elderly 18 months old rats.

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