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Specificity of Piracetam's Anti-Amnesic Activity in Three Models of Amnesia in the Mouse

A LENÈGRE,* R. CHERMAT,† I AVRIL,* L. STÉRU* AND R. D PORSOLT*[1]

*I T E.M.-Labo, 93 ave de Fontainebleau, 94270 Kremlin-Bicêtre, France
†Faculté de Médecine Pitié-Salpétrière, 75013 Paris, France

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LENÈGRE, A , R CHERMAT, I AVRIL, L STÉRU AND R D PORSOLT *Specificity of piracetam's anti-amnesic activity in three models of amnesia in the mouse* PHARMACOL BIOCHEM BEHAV **29**(3)625–629, 1988 —The effects of piracetam on the amnesias induced by scopolamine, diazepam and electroconvulsive shock (ECS) were studied in a passive avoidance procedure in the mouse and compared with the interactions of piracetam with the major behavioral effects of these treatments, namely scopolamine-induced hyperactivity, diazepam-induced release of punished behavior (Four Plates Test) and ECS-induced convulsions Amnesia was induced by injecting scopolamine or diazepam (1 mg/kg, IP) 30 minutes before or applying ECS immediately after the first session (S1) of the passive avoidance task Piracetam was studied at 3 doses (512, 1024 and 2048 mg/kg) administered PO 60 minutes before S1 Retention was measured 24 hours later (S2) in the absence of any treatment Piracetam dose-dependently attenuated the memory deficits induced by the three amnesic treatments but did not affect either scopolamine-induced hyperactivity, diazepam-induced release of punished behavior or ECS-induced convulsions These results point to the specificity of piracetam's anti-amnesic activity and, in particular, suggest that piracetam can suppress the memory disturbances induced by diazepam without affecting diazepam's anxiolytic activity The test battery employed would therefore seem highly suitable for evaluating the potential nootropic activity of novel compounds

Piracetam Amnesia Passive avoidance Scopolamine Diazepam Electroconvulsive shock Specificity of action Nootropic drugs

EXPERIMENTAL amnesia can be induced in animals by a variety of treatments including electroconvulsive shock [7], anticholinergic agents [5] and benzodiazepines [13] Antagonism of different experimental amnesias has provided the major approach towards the development of drugs useful for treatment of memory problems in old age [3,11]. The predictive value of such models is by no means well established but it seems reasonable to suppose that a compound which antagonizes amnesias of different origins is more likely to be generally effective Furthermore, antagonism by the same compound of different kinds of amnesias provides evidence for the specificity of the anti-amnesic activity of the compound as opposed to its effects on one or more systems [8]

The term nootropic was suggested by Giurgea [4] for classifying drugs which enhance memory and attenuate experimental amnesia, the prototype drug in this category is piracetam. The experiments described in the present paper investigated the antagonism by piracetam of amnesias induced by scopolamine, diazepam and electroconvulsive shock (ECS) and then examined the interaction of piracetam with the major behavioral effects of these three amnesic treatments, namely the hyperactivity induced by scopolamine, the release of punished behavior by diazepam and the convulsions induced by ECS The rationale for choosing amnesias of three different origins in the same test situation was that an antagonism by piracetam of all three amnesias would rule out interpretations of piracetam's effects in terms of a specific antagonism of the intrinsic properties of any one of the amnesic agents. Such a conclusion would be comforted by demonstrating the absence of antagonism by piracetam of the other behavioral consequences of the amnesic treatments

METHOD

Animals

The subjects were male NMRI mice, weighing 23–25 g, supplied by the Centre d'Elevage Roger Janvier (CERJ), 53940 Le Genest Saint Isle, France They were housed in groups of 10 in transparent macrolon cages (25 5×19 5×13 5 cm) containing sawdust with free access to food (UAR 113) and tap water All animals were delivered to the laboratory

[1]Requests for reprints should be addressed to Dr R D Porsolt, Scientific Director, ITEM-Labo, 93 ave de Fontainebleau, 94270 Kremlin-Bicêtre, France

at least 3 days before being used in an experiment and were kept in an ambient temperature of 21±1°C under artificial lighting (12 hours) between 8 00 and 20 00

Drugs

The following drugs were used diazepam (Roche), piracetam (UCB) and scopolamine hydrobromide (Sigma) They were either dissolved in distilled water (piracetam, scopolamine) or dispersed in an aqueous suspension of acacia gum (5%) (diazepam) All drugs were injected in a volume of 0 25 ml/20 g body weight Doses are expressed in terms of the base or salt where appropriate

Procedure

Passive avoidance tests Mice were placed individually into the smaller (10×10×29 cm), but brightly lit, compartment of a two compartment box When they crossed with all four paws into the larger darker compartment (19 5×16 5×29 cm) they received a light foot-shock (0 35 mA) until they returned to the lighted compartment from which they were immediately removed (S1) Twenty-four hours later they were replaced in the lighted compartment without any pretreatment and their latency before crossing to the dark compartment was measured with a cut-off time of 180 seconds (S2) Twenty mice were used per group

In the experiments where amnesia was induced by scopolamine mice were given an IP injection of scopolamine (1 mg/kg) 30 minutes before S1 In the experiments where amnesia was induced by diazepam mice were given an IP injection of diazepam (1 mg/kg) 30 minutes before S1 In the experiments where amnesia was induced by ECS mice were given ECS (rectangular current, 0 4 sec, 50 mA, 50 Hz, via temporal electrodes connected to a Ugo Basile Shock Generator) on removal from the apparatus immediately after S1

In all amnesia experiments piracetam (512, 1024 and 2048 mg/kg) was given in a single PO administration 60 minutes before S1 In each experiment two control groups were employed Normal controls, instead of receiving the amnesic agent, received IP injections of distilled water (scopolamine) or 5% acacia gum (diazepam) or simple placement of the electrodes without shock (ECS) Controls (normal and amnesic), instead of receiving piracetam, were given an oral administration of distilled water 60 minutes before S1

Spontaneous motor activity For investigating the interaction of piracetam with the motor stimulatory effects of scopolamine a photo-cell activity meter similar to that described by Boissier and Simon [2] was used The activity meter consisted of 6 covered Plexiglas enclosures (25 5×20 5×9 cm) each equipped with two criss-cross photo-cell assemblies contained within a darkened enclosure. The number of interruptions of the photo-electric beams by each animal was counted during a 30 minute test Twelve animals were studied per group

The experiment consisted of four groups control, piracetam (2048 mg/kg) alone, scopolamine (1 mg/kg) alone, piracetam (2048 mg/kg)+scopolamine (1 mg/kg) Piracetam was administered PO 60 minutes before the test and scopolamine was administered IP 30 minutes before the test All animals received two injections, when a drug was not given they received the vehicle

Four Plates Test For investigating the interaction of piracetam with the anxiolytic effects of diazepam the Four Plates Test [1] was used Mice were placed individually in a white plastic enclosure (25×18×16 cm) with a floor consisting of four rectangular metal plates (8×11 cm) The animal was left to explore freely for 15 sec and then for the next 60 sec it received a mild electric shock (0 35 mA, 0 5 sec) every time it crossed from one plate to another The number of punished crossings during this period was counted Ten animals were studied per group

The experiment consisted of four groups control, piracetam (2048 mg/kg) alone, diazepam (2 mg/kg) alone, piracetam (2048 mg/kg)+diazepam (2 mg/kg) Piracetam was administered PO 60 minutes before the test and diazepam was administered IP 30 minutes before the test All animals received two injections, when a drug was not given they received the vehicle

Electroconvulsive shock (ECS) For investigating the interaction of piracetam with the effects of ECS, the number of animals showing full tonic-clonic convulsions in the different experimental groups after administration of ECS on the first day (S1) of the passive avoidance task was counted There were thus 20 animals per group

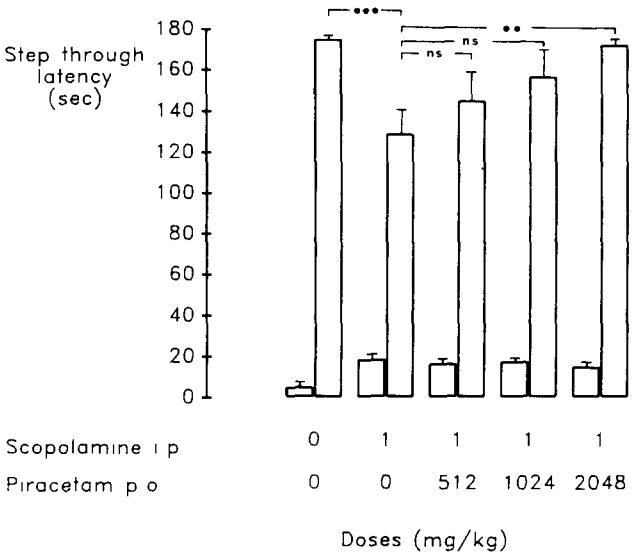

FIG 1 Scopolamine-induced amnesia The effects of piracetam, administered PO 60 minutes before the first session (S1) on the mean (±s e m) step-through latency at S1 (left columns) and S2 (right columns) of a passive avoidance task Twenty mice were used per group All animals, except the non-amnesic control group, received an IP injection of scopolamine (1 mg/kg) 30 minutes before S1 **=$p<0$ 01, ***=$p<0$ 001 (unpaired Student's t-test, two-tailed)

Statistical Tests

All quantitative results were analyzed for statistical significance using the Student's t-test for independent samples (two-tailed)

RESULTS

Passive Avoidance Tests Amnesias Induced by Scopolamine, Diazepam and ECS

The effects of piracetam on scopolamine-induced amnesia are shown in Fig 1 Control mice which had received only injections of distilled water (normal controls) showed clear retention as indicated by the close to maximal step-through latencies at S2 Control mice which had received an IP injection of scopolamine 30 minutes before S1 showed a clear and

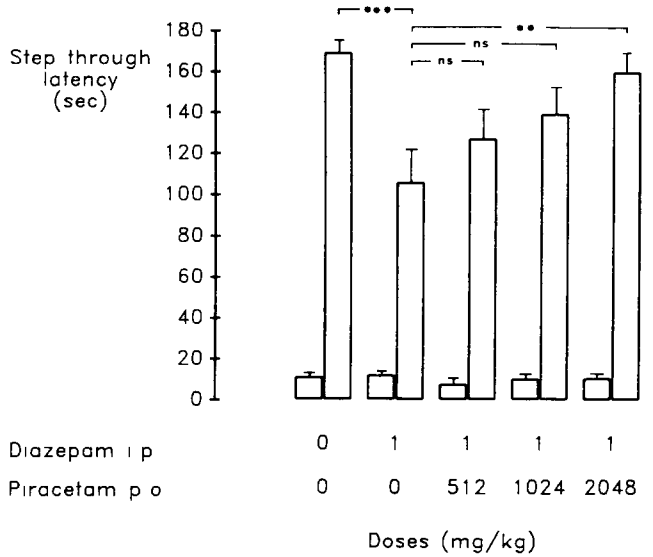

FIG 2 Diazepam-induced amnesia The effects of piracetam, administered PO 60 minutes before the first session (S1) on the mean (±s e m) step-through latency at S1 (left columns) and S2 (right columns) of a passive avoidance task Twenty mice were used per group All animals, except the non-amnesic control group, received an IP injection of diazepam (1 mg/kg) 30 minutes before S1 **=$p<0$ 01, ***=$p<0$ 001 (unpaired Student's t-test, two-tailed)

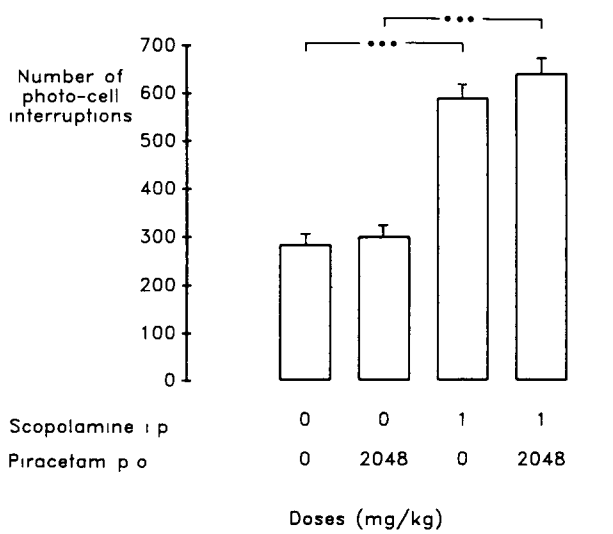

FIG 4 Interaction of piracetam with scopolamine-induced hyperactivity The effects of scopolamine and piracetam either alone or administered together on the mean (±s e m) number of photo-cell beams interrupted during a 30 minute session Piracetam (2048 mg/kg) was administered PO 60 minutes before testing and scopolamine (1 mg/kg) was administered IP 30 minutes before testing Twelve mice were used per group ***=$p<0$ 001 (unpaired Student's t-test, two-tailed)

significant decrease in S2 latencies as compared with the normal controls indicating scopolamine-induced amnesia. Piracetam administered PO 30 minutes before scopolamine at S1 had no effect on S1 latencies but caused a dose-dependent increase in S2 latencies suggesting antagonism of scopolamine-induced amnesia which was almost complete at the highest dose tested (2048 mg/kg PO)

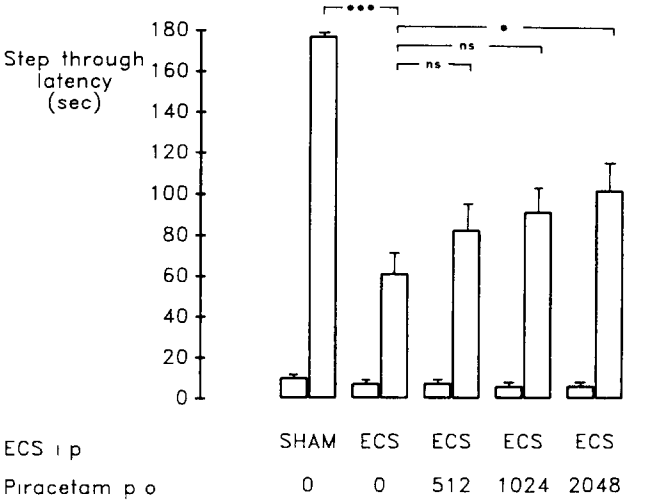

FIG 3 ECS-induced amnesia The effects of piracetam, administered PO 60 minutes before the first session (S1) on the mean (±s e m) step-through latency at S1 (left columns) and S2 (right columns) of a passive avoidance task Twenty mice were used per group All animals, except the non-amnesic control group, received ECS immediately after S1 *=$p<0$ 05, ***=$p<0$ 001 (unpaired Student's t-test, two-tailed)

The effects of piracetam on diazepam-induced amnesia are shown in Fig 2 Like in the scopolamine experiment diazepam, administered IP 30 minutes before S1, caused a marked and significant decrease in S2 latencies as compared with the normal control group indicating diazepam-induced amnesia Piracetam, administered PO 30 minutes before diazepam, had no effect on S1 latencies but caused a clear and a dose-dependent increase in S2 latencies suggesting, as in the scopolamine experiment, antagonism of diazepam-induced amnesia which was virtually complete at the highest dose tested (2048 mg/kg PO)

The effects of piracetam on ECS-induced amnesia are shown in Fig 3 ECS, administered to vehicle treated animals immediately after S1, caused a marked decrease in S2 latencies as compared to sham treated control animals indicating ECS-induced amnesia. The decrease in S2 latencies after ECS appeared to be rather more marked than that observed in the scopolamine or diazepam experiments Piracetam, administered PO 60 minutes before S1, caused a dose-dependent increase in S2 latencies, suggesting as in the previous two experiments, antagonism of ECS-induced amnesia. Although the same doses of piracetam were investigated, the antagonism by piracetam of ECS-induced amnesia appeared to be less complete than that observed in the scopolamine and diazepam experiments

Spontaneous Motor Activity Interaction Between Piracetam and Scopolamine

The effects of scopolamine and piracetam alone or administered together on spontaneous motor activity are shown in Fig 4 Scopolamine (1 mg/kg IP) alone caused a marked increase in the number of photo-cell beams interrupted by mice placed in the activity meter Piracetam, at the dose which was active in antagonizing scopolamine-induced amnesia (2048 mg/kg PO), was itself without significant effects on spontaneous motor activity and did not significantly modify scopolamine-induced hyperactivity

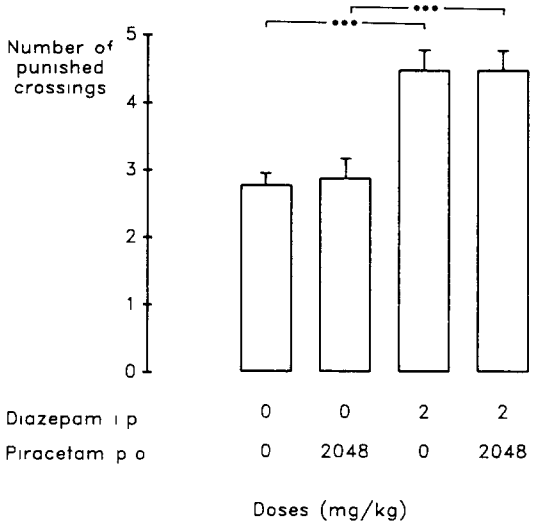

FIG 5 Interaction of piracetam with diazepam-induced release of punished responding The effects of diazepam and piracetam either alone or administered together on the mean (±s e m) number of punished crossings during the Four Plates Test Piracetam (2048 mg/kg) was administered PO 60 minutes before testing and diazepam (2 mg/kg) was administered IP 30 minutes before testing Ten mice were used per group ***=$p<0$ 001 (unpaired Student's t-test, two-tailed)

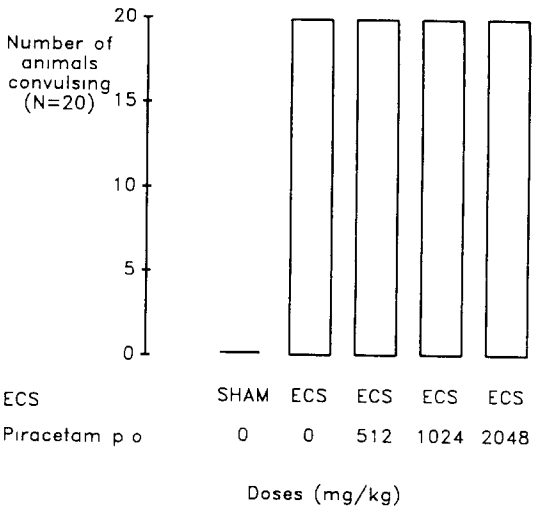

FIG 6 Interaction of piracetam with convulsions induced by electroshock The effects of three doses of piracetam on the number of animals convulsing after application of ECS through temporal electrodes Twenty mice were used per group

Four Plates Test Interaction Between Piracetam and Diazepam

The effects of diazepam and piracetam alone or administered together on the number of punished crossings are shown in Fig 5 Diazepam (2 mg/kg IP) alone caused a significant increase in the number of punished crossings reflecting its anxiolytic activity in this test Piracetam, at the dose which was active in antagonizing diazepam-induced amnesia (2048 mg/kg PO), was itself without significant effect on the number of punished crossings and did not significantly modify the increase in punished crossings observed with diazepam

Convulsions Induced by ECS Interaction With Piracetam

The effects of the three doses of piracetam used in the amnesia experiment on the convulsions induced by ECS are shown in Fig 6 No convulsions were observed in sham treated animals whereas all control animals receiving ECS showed full tonic-clonic convulsions Piracetam did not protect against electrically-induced convulsions at any of the doses tested

DISCUSSION

The present experiments have shown that piracetam antagonizes the amnesias induced by three different amnesic treatments, scopolamine, diazepam and ECS These results confirm the proposed nootropic profile of this compound [4] and suggest that its anti-amnesic effects are unrelated to the specific nature of these amnesia-inducing agents This conclusion is supported by the finding that piracetam in no way interacts with the major behavioral effects of these treatments namely the hyperactivity induced by scopolamine, the anti-punishment effect of diazepam or the convulsant effect of electroshock The anti-amnesic effects of piracetam occur at doses of this compound which have no observable effects on behavior either during the passive avoidance test itself or on any of the other behaviors investigated in the present experiments The anti-amnesic effects observed, moreover, occur in animals which are tested at S2 in the absence of the compound thereby ruling out possible interpretations in terms of the effects of piracetam on performance or even state-dependent effects [9]

Although these experiments clearly suggest a specific anti-amnesic action of piracetam, the absence of other effects provide no clue as to its mechanism of action The compound exerts no clear effects on any brain neurotransmitter system and, in particular, appears to be devoid of classical cholinergic activity [6] although piracetam has been reported to act synergistically with the acetylcholine precursor choline in facilitating memory function in rodents [10] Absence of direct cholinergic activity is also indicated in the present experiments by piracetam's failure to antagonize scopolamine-induced hyperactivity Furthermore, although structurally related to GABA it did not interact with diazepam's anxiolytic activity nor possess convulsant or anticonvulsant activity as might be expected of substances interacting with GABAergic neurotransmission [12] It is possible that different thresholds exist for different behaviors and that piracetam antagonizes the memory disrupting effects of different amnesic treatments without blocking their overt behavioral effects For example, piracetam could conceivably attenuate some of the disruption of CNS activity that presumably mediates amnesia but fail to block overt convulsions This seems an unlikely explanation for all the results obtained in the present experiments however, particularly where it was shown that diazepam, which is a potent anticonvulsant, itself induced amnesia

From a more pragmatic point of view, the present results suggest the usefulness of the test battery employed for evaluating the anti-amnesic activity of piracetam or other compounds potentially useful for treating memory disturbance Furthermore it may be of practical significance that piracetam could be shown to antagonize diazepam-induced amnesia without apparently affecting diazepam's anxiolytic activity

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