Memory Storage and Effect of Repeated Treatment with a New Antidepressant Drug: Rubidium Chloride

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Following 15 days' treatment with saline, 48 mg/kg rubidium chloride, 5 mg/kg imipramine hydrochloride, 10 mg/kg sodium phenobarbitone, 1000 mg/kg piracetam, or 0.20 mg/kg strychnine nitrate all administered intraperitoneally, mice were evaluated by habituation of exploratory activity using an open-field apparatus. In control animals a significant \( (P < 0.05) \) decrease in open-field responses (ambulation, rearing and defaecation) was seen following a 1-day intersession interval and there was no retention of exploratory activity after a 5-day intersession interval. Administration of imipramine or phenobarbitone for 15 days was found to impair retention of memory after 1 day, whereas treatment with rubidium chloride, piracetam, or strychnine for 15 days improve retention after a 5-day intersession interval.

KEY WORDS: Rubidium chloride; piracetam; strychnine; imipramine; phenobarbitone; memory storage; exploratory activity.

INTRODUCTION

It is generally believed that the antimuscarinic properties of tricyclic antidepressant drugs reflect their ability to bring about peripheral and central side-effects. In particular, much attention has centred on the antimuscarinic and sedative properties of those drugs that have been implicated in the impairment of memory and cognition in humans.1,2

Rubidium chloride, although its basic pharmacology has been well established,3 has only recently been introduced as an antidepressant drug for the treatment of humans. Biochemical studies have demonstrated that rubidium chloride enhances the turnover of noradrenaline in rats in whom biosynthesis of the catecholamine had been inhibited.4 Furthermore, in contrast to the effects of lithium in both humans and animals, rubidium chloride has been found to increase physical activity and alertness in monkeys.5

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and ambulation and rearing in rats. These findings are in contrast with those of another report in which a decrease in both measures of exploratory activity was demonstrated.

In light of previous information, the present experiments were designed to determine and compare the effects of rubidium chloride with those of memory-impairing (imipramine, phenobarbitone) or memory-enhancing (piracetam, strychnine) drugs in an experimental model of memory, i.e. habituation of exploratory activity in mice. The therapeutic effects of imipramine and rubidium chloride do not develop until 10–14 days after initiation of therapy; therefore, experiments were performed 14 days after the daily administration of the drugs.

MATERIALS AND METHODS

Animals

A total of 60 female Charles River CD1 mice (body weight 22–24 g) were used. They were housed in groups of 10 in opaque Perspex boxes under standard laboratory conditions (non-reversed 12 h light/dark cycle, relative humidity 50–60%, temperature 21 ± 2°C) with free access to food and tap water.

Treatment

Rubidium chloride (Aldrich, USA), imipramine hydrochloride (Geigy, Italy), piracetam (Cerebropan®), strychnine nitrate (Sigma, USA) and sodium phenobarbitone (Fluka, Switzerland) were dissolved in vehicle saline (0.9% w/v sodium chloride). Rubidium chloride (48 mg/kg), 5 mg/kg imipramine hydrochloride, 1000 mg/kg piracetam, 0.2 mg/kg strychnine nitrate and 10 mg/kg sodium phenobarbitone were administered intraperitoneally once daily for 14 days in a volume of 0.01 ml/g body weight. The doses were chosen from preliminary dose–response experiments and are expressed in terms of salt or base where appropriate. There were 10 mice in each treatment group and the drugs were coded so that the two observers scoring the open-field tests had no knowledge of the drug treatment of any mouse.

Open-field testing

Behavioural patterns (ambulation, rearing and defaecation) were assessed using an open-field apparatus. A painted wooden box (60 x 60 x 30 cm) was divided in 36 squares each 10 x 10 cm. The apparatus was lit by a 100 W light above the floor of the box. An additional 60 W lamp was placed above the centre of the apparatus and 150 cm from the floor of the box. The two observers sat on opposite sides of the box at a height of 150 cm and a distance of 60 cm from the centre of the box. At the end of each session any boli were removed and the floor and walls of the box were wiped with detergent and dried.

Mice were given two sessions in the open-field apparatus and the decrease in activity during the second session (habituation) served as index of memory retention. An acquisition session consisted of placing each mouse on its own in the open-field apparatus and scoring behavioural activity over a 3-min period. The retention session carried out 1 or 5 days after the acquisition session was the same except that no injection was given. The following behavioural parameters were measured: ambulation, the number of squares the animal crossed during the 3-min observation period; rearing, the number of times the animal stood on its hind feet in the 3-min observation period; and defaecation, the number of faecal boli the animal produced in the 3-min observation period. After treatment with the test drug or vehicle injections for 14 days, on day 15 the acquisition session was carried out and a single test injection of the drug or vehicle was given immediately after the acquisition session. The experiments were performed

Cerebropan® is a registered tradename of Istituto Sieroterapico Milanese, Italy.
between 09.30 h and 11.30 h.

Statistical analysis
Data were subjected to analysis of variance. In saline-treated control mice, the significance of differences between the acquisition sessions and retention sessions was determined using paired Student’s t-test. In drug-treated animals, the mean of the difference in scores between the acquisition sessions and retention sessions was compared with the same measure for the saline-treated animals using unpaired Student’s t-test. Differences were considered significant at $P < 0.05$.

RESULTS
In saline-treated control mice, a significant ($P < 0.05$, paired Student’s t-test) decrease between acquisition and retention session ambulatory scores was seen when the intersession interval was 1 day ($155.40 \pm 8.22$ compared with $118.00 \pm 5.02$, Fig. 1a), whereas the difference between scores when there was a 5-day intersession interval failed to reach significance ($158.10 \pm 10.23$ compared with $162.80 \pm 12.01$, Fig. 1b). In the mice receiving repeated administration of imipramine or phenobarbitone the difference in the ambulatory activity between the acquisition and retention sessions did not vary significantly after either a 1- or 5-day intersession interval. When mice had been treated with rubidium chloride, piracetam, or strychnine, a significant ($P < 0.05$, unpaired Student’s t-test) decrease in the 5-day intersession activity compared with saline-treated mice was detected, suggesting a facilitation of memory retention (Fig. 1b).

With regard to rearing, in mice receiving saline, a significant ($P < 0.05$; paired Student’s t-test) decrease between acquisition and retention scores was found when the intersession interval was 1 day ($20.30 \pm 1.31$ compared with $15.00 \pm 0.87$, Fig. 2a). In contrast, when control mice were tested in the open-field apparatus 5 days after the acquisition session, no significant difference between the two sessions was seen ($22.00 \pm 1.80$ compared with $24.00 \pm 1.72$, Fig. 2b). When mice were treated with strychnine, imipramine, or phenobarbitone, the intersession decrease in comparison with control animals at 1 day was abolished (Fig. 2a). In the case of the 5-day intersession interval, however, the mean difference in scores between the two sessions for rubidium chloride, piracetam and strychnine compared with the same measures for the control group was significant ($P < 0.05$, unpaired Student’s t-test; Fig. 2b).

In saline-treated control mice, a significant ($P < 0.05$, paired Student’s t-test) decrease in the frequency defaecation was found when the intersession interval was 1 day ($1.61 \pm 0.14$ compared with $0.63 \pm 0.05$, Fig. 3a). As observed for ambulatory activity and rearing, saline-treated control mice showed no memory retention based on frequency of defaecation after a 5-day intersession interval. Furthermore, as seen in Figs 1a and 2a, retention after 1 day was impaired in animals chronically treated with imipramine. With respect to pheno­barbitone, a significant ($P < 0.05$, unpaired Student’s t-test) decrease in defaecation scores was detected with both 1- and 5-day intersession intervals (Figs 3a and b). After a 5-day intersession interval (Fig. 3b), mice given rubidium chloride, piracetam, or strychnine also showed a significant ($P < 0.05$, unpaired Student’s t-test) decrease in defaecation when compared to saline-treated animals.

DISCUSSION
In agreement with previous findings in other animal models, the results of the present study indicate that retention of the open-field response in saline-treated mice was good when the interval between the acquisition and retention sessions was 1 day and decreased as the interval between sessions was increased to 5 days. In fact, a
Fig. 1. Effects of intraperitoneal administration of 48 mg/kg rubidium chloride, 1000 mg/kg piracetam, 5 mg/kg imipramine hydrochloride, 0.2 mg/kg strychnine nitrate, 10 mg/kg sodium phenobarbitone, or saline daily for 15 days on memory storage measured by ambulatory activity (number of squares the mouse crossed in the 3-min observation period) with an interval of (a) 1 day or (b) 5 days between memory acquisition and retention sessions; *$P < 0.05$ compared with acquisition session.
Rubidium chloride in memory storage

Fig. 2. Effects of intraperitoneal administration of 48 mg/kg rubidium chloride, 1000 mg/kg piracetam, 5 mg/kg imipramine hydrochloride, 0.2 mg/kg strychnine nitrate, 10 mg/kg sodium phenobarbitone, or saline daily for 15 days on memory storage measured by rearing activity (number of times the mouse stood on its hind feet in the 3-min observation period) with an interval of (a) 1 day or (b) 5 days between memory acquisition and memory retention sessions; *P < 0.05 compared with acquisition session.
Fig. 3. Effects of intraperitoneal administration of 48 mg/kg rubidium chloride, 1000 mg/kg piracetam, 5 mg/kg imipramine hydrochloride, 0.2 mg/kg strychnine nitrate, 10 mg/kg sodium phenobarbitone, or saline daily for 15 days on memory storage measured by defaecation (number of faecal boli produced in the 3-min observation period) with an interval of (a) 1 day or (b) 5 days between memory acquisition and retention sessions; $P < 0.05$ compared with acquisition session.
significant decrease in ambulatory activity, rearing and defaecation was observed in saline-treated control mice when the intersession interval was 1 day and a poor retention was observed when the intersession interval was 5 days. This time-dependent effect in mice chronically treated with saline agreed with previous findings that investigated acute post-session treatment. Present data on ambulatory scores showed that chronic administration of rubidium chloride, as well as drugs that are known to enhance learning, i.e. piracetam and strychnine, improved the poor retention at the 5-day intersession interval.

Of interest is the finding that habituation of open-field responses after 1 day was impaired by repeated administration of imipramine. The cholinergic system has long been thought to play an important role in memory processes and the anticholinergic properties of imipramine must be borne in mind. In particular, the blockade of central muscarinic receptors is commonly known to impair memory in man and a decrease of central cholinergic transmission has been attributed to memory dysfunction in geriatrics.

When considering the rearing response, it may be observed that, after 1- or 5-day intersession intervals, chronic administration of rubidium chloride, piracetam, or strychnine interfered with curiosity tendencies almost to the same extent as the effect on ambulatory activity.

Furthermore, the fact that after a 5-day intersession interval mice given rubidium chloride, piracetam, or strychnine showed a significantly greater decrease in defaecation scores than saline-treated animals may indicate that there is a relationship between emotional reactivity and drug treatment. In fact, animal reactivity, which may be considered as an index of sensitivity to stress situations, has been operationally defined as the number of faecal boli produced by the animal when placed in a novel environment, i.e. the open-field apparatus. This suggests that in these animals there may have been a facilitation of memory retention and, therefore, after a 5-day intersession interval, they behaved in a less fearful and emotional manner than saline-treated animals. That fact that animals treated chronically with phenobarbitone also showed a significant decrease in defaecation compared with saline-treated mice, both at 1- and 5-day intersession intervals, may be correlated with the anxiolytic properties of this drug. The absence of significant differences in ambulatory and rearing scores between phenobarbitone- and saline-treated animals suggests that the sedative properties of phenobarbitone were not important at the dose used in the present study.

It is concluded that habituation of open-field activity in mice provides a useful and simple method for the preclinical evaluation of memory-impairing or memory-enhancing drugs.

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REFERENCES


