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Microtubule Disruption and Cognitive Defects: Effect of Colchicine on Learning Behavior in Rats

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BENSIMON, G. AND R. CHERMAT. *Microtubule disruption and cognitive defects: Effect of colchicine on learning behavior in rats.* PHARMACOL BIOCHEM BEHAV **38**(1) 141–145, 1991.—Neuropathological findings in Alzheimer's disease (AD) suggest a possible involvement of microtubule dysfunction in neurodegenerative process pathogenesis. Because microtubules have a major role in neuronal plasticity, microtubule disruption could be also directly responsible for cognitive defects in AD. We report that in rats, continuous microtubule disruption induced by chronic colchicine administration results in a dose-dependent learning deficit. In addition, retention is also impaired. These cognitive defects are specific, as chronic colchicine induces no other behavioral toxicity within the study dose range. Colchicine-induced cognitive defects resemble those of AD, which are characterised by amnesia of recent learning and loss of formerly established memories. This new procedure of pharmacologically induced cognitive impairment may prove useful, both towards understanding AD pathogenesis and towards drug screening.

Memory Alzheimer's disease Microtubule Animal model Operant conditioning Colchicine Dose-effect

ALZHEIMER'S disease (AD) was shown to be associated with microtubule dysfunction (10, 13, 16, 21). Typical histological lesions in AD include neurofibrillary tangles and amyloid-containing neuritic plaques (14), one of their major constituents being a defective microtubule-associated protein (tau) (15, 17, 26). Density of neurofibrillary tangles and neuritic plaques in AD correlates with the severity of cognitive impairment (24,33). Common interpretation of the link between cognitive impairment and microtubule dysfunction postulates cerebral cell loss. Indeed, under experimental conditions in animals, intracranial administration of microtubule-disrupting drugs can result in cell death, associated with some cognitive impairment (11, 23, 27–30). However, the cytotoxic effect of microtubule-disrupting drugs is cell-specific and does not topographically fit with the extensive cortical and subcortical cell depletion present in AD. Furthermore, these lesions do not succeed in reproducing the main cognitive defect of AD, i.e., the memory loss of well-acquired knowledge (32).

We propose alternatively that microtubule dysfunction per se is responsible for the cognitive defect in AD. The assumption that long-lasting memory engrams are ultimately related to plastic changes in neuronal shape, arborisation and connection has now received consistent experimental support (8, 9, 31, 36). Since besides supporting axoplasmic transport, microtubules are critically involved in cell shape and neuritic growth control (22), this last function could be relevant to memory process, and its disruption to cognitive defect in AD.

In the following experiments we investigated the behavioral effects of continuous microtubule disruption in rats. Systemic colchicine was chronically administered at doses that have been shown to result in nonlesioning, though active, concentrations in the central nervous system (3,5). Behavioral assessments were specially focused on 1) long-term memory formation, 2) retention of a well-acquired behavior, 3) some possible nonspecific effects.

METHOD

Animals

Animals were male Wistar AF rats (CERJ, Le Genest-Ste-Isle, France), weighing 80–100 g at the beginning of the experiments and maintained at 80–85% of their free-feeding body weight by daily feeding with measured rations. They were housed 10 per cage under standard conditions (12-h light-dark cycle; room temperature 21 ± 1°C) with water freely available in the home cage.

Drugs

Colchicine (15, 30, 60 and 120 μg/kg; Houdé Laboratory, France) and β-lumicolchicine (60 μg/kg; Sigma Chemical, St. Louis, MO) were dissolved in saline just before use; treatments were injected intraperitoneally (IP) 90 min before tasks.

Behavioral Evaluations

Operant conditioning. Rats were subjected to 10 daily 15-

minute learning sessions of lever pressing for food, according to a continuous reinforcement schedule (CRF = each lever press was reinforced by one food pellet) (25). During the learning session, rats were required to press the right lever of an operant chamber (Campden Instrument Ltd.) to obtain food pellets (45 mg, Campden); pressing the left lever had no scheduled consequences. The operant chamber (housed in ventilated sound-attenuated cubicles) was equipped with levers (5.5 cm above the floor) which required a vertical force of at least 12 g to operate the microswitch. Rewards were delivered into a recess between the two levers, in front of which was a 5 cm wide transparent flap the rat had to push open with its nose to collect pellets. The number of lever presses, flap openings and pellets consumed was recorded.

Locomotor activity. Locomotor activity was measured using a photocell actimeter chamber (27 × 22 × 11 cm) equipped with two counters mounted at 90° across the box (4). Rats were placed individually in the actimeter 90 min after drug administration and the number of light beam interruptions was counted over a 30-min session.

Statistics

Learning. Overall effect of drugs was tested with ANOVA for repeated measures (34), including a drug effect as between factor and a session effect as the within factor (repeated measure); the interaction drug × session was considered to test for drug effect on learning. Learning curves were also summarized by their slopes and by the performance at the end of the learning procedure; learning curve slope was calculated for each rat, using a simple linear regression model, and was used as an independent variable. Dose-effect relationships on learning slopes, and performance during the last session were evaluated with a simple linear regression model.

Locomotor activity. Overall drug effect was tested with one-way variance analysis.

EXPERIMENT 1

Dose-Effect Relationship of Colchicine on Learning

The purpose of this experiment was to study the dose-effect relationship of colchicine administration on learning rates in the operant paradigm. Four groups of animals (n = 10/group) were given colchicine (15, 30, 60 or 120 μg/kg). Each treated group was matched with an independent control group of rats (n = 10) given saline (0.5 ml/100 g body weight).

RESULTS

The number of lever-presses performed by the 4 control groups progressively increased over the experimental sessions to reach an asymptotic value between the 7th and the 10th session (Fig. 1, A–D). Analysis of variance (ANOVA) showed that, compared to control, the first studied dose (15 μg/kg) already induced a slight but significant learning impairment [drug × session interaction: $F(9,162) = 7.14$, $p<0.0001$]. A maximum response suppression was obtained with the highest dose (120 μg/kg) [drug × session interaction: $F(9,162) = 38.79$, $p<0.0001$].

A linear dose-effect relationship was found on performances obtained during the 10th session as well as on learning slope [$Y = 101.5 - (0.84 \pm 0.08) \times$ Dose; $F(1,78) = 104.5$, $p<0.0001$ and $Y = 12.22 - (0.10 \pm 0.01) \times$ Dose; $F(1,78) = 110.2$, $p<0.0001$, respectively] (Fig. 1, E, F). Thus chronically administrated colchicine reliably impaired rats' ability to learn pressing a lever for food.

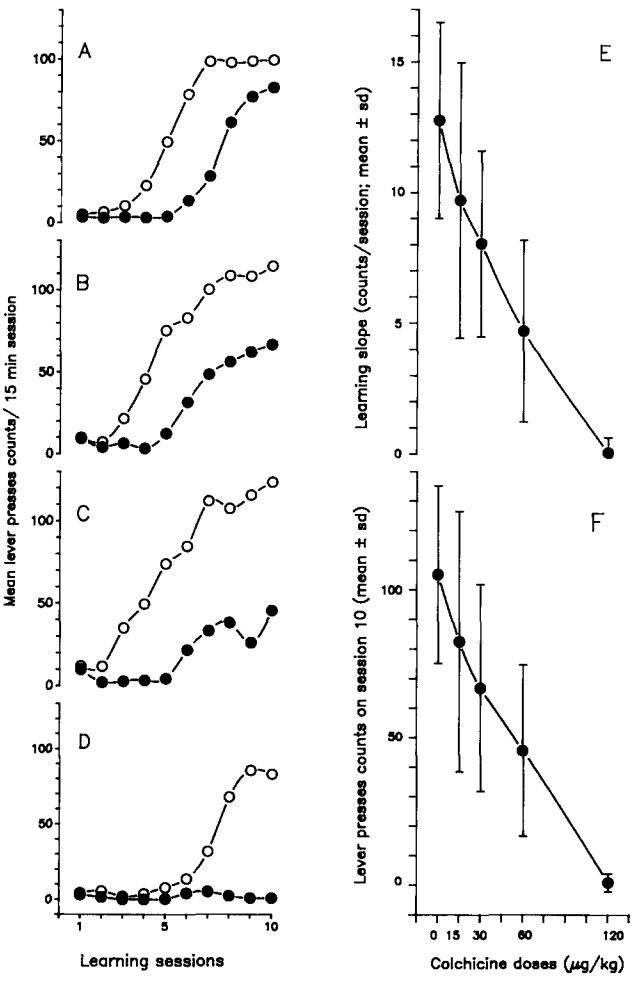

FIG. 1. Dose-effect relationships of chronic colchicine (15, 30, 60, 120 μg/kg intraperitoneally 90 min before test) on a food-rewarded operant conditioning: lever press counts per 15-min session. A to D: Acquisition curves of colchicine doses (●) (A = 15 μg/kg, D = 120 μg/kg; n = 10 per group); compared to saline control (○) (n = 10 per group) all doses induced a learning impairment [ANOVA for repeated measures; $p<0.0001$]. E and F: Dose-effect relationships on learning slope (E) and on final performance (F). Simple linear regressions show a significant linear dose-effect relation on both parameters, $F(1,78) = 110.2$, $p<0.0001$ and $F(1,78) = 101.5$, $p<0.0001$.

EXPERIMENT 2

Effect of Chronic Colchicine on Locomotor Activity

Although no other clear, overt behavioral change or sickness, evaluated with a classical screening procedure (18), was observed in any additional experiments (data not shown), the possibility of an incapacitating effect of chronic colchicine was investigated in this experiment. Three groups of rats (n = 10/group) were given either colchicine (60 or 120 μg/kg), or saline IP once a day for ten days. Locomotor activity of rats was evaluated 90 min after the last administration.

RESULTS

At the studied doses, chronic colchicine administration did not induce any significant variation of locomotor activity [locomotor activity (mean ± sd): saline = 115.6 ± 13.9; colchicine (60 μg/kg) = 101.1 ± 15.3; colchicine (120 μg/kg) = 124.0 ± 24.4; one-way ANOVA: $F(2,27) = 0.394$, NS]. Thus the reduced performances during the learning procedure cannot be accounted

for by a motor incapacitation induced by chronic colchicine.

EXPERIMENT 3

Comparison of Colchicine and β-Lumicolchicine Effects on Learning

To control the role of microtubule disruption in the colchicine-induced learning impairment, the effects of this compound were compared to those of β-lumicolchicine. β-Lumicolchicine is a structural isomer of colchicine which neither binds tubulin nor interferes with microtubule assembly. However, β-lumicolchicine displays membrane-related activity similar to that of colchicine, particularly on the cholinergic nicotinic receptor complex (19,20). In this experiment, three groups of rats (n = 10/group) were given saline, colchicine (60 μg/kg) or β-lumicolchicine (60 μg/kg) IP before undergoing the learning procedure.

RESULTS

As expected, colchicine (60 μg/kg) induced a significant response suppression on final performances [lever presses (mean ± sd): saline = 105.2 ± 20.7 vs. colchicine = 31.3 ± 41.6; $t(18df, 2\alpha) = 3.56$, $p<0.005$] and a significant decrease on learning rates [slope (mean ± sd): saline = 11.3 ± 2.8 vs. colchicine = 5.3 ± 3.7; $t(18df, 2\alpha) = 2.90$, $p<0.01$]. Conversely, β-lumicolchicine (60 μg/kg) did not significantly modify these learning parameters as compared with control [lever presses (mean ± sd) = 106.4 ± 14.9; $t(18df, 2\alpha) = 0.10$, NS; slope (mean ± sd) = 11.9 ± 2.0; $t(18df, 2\alpha) = 0.38$, NS]. These results demonstrate clearly that colchicine-induced learning impairment is related to microtubule disruption and not to a potential anticholinergic activity of the drug.

EXPERIMENT 4

The final experiment was done to give some insight into the possible biological consequences of colchicine-induced microtubule disruption that could explain the learning impairment. One commonly held view is that neuronal cell loss is responsible for the learning deficit. As in lesioning models then, the learning deficit should persist long after the discontinuation of colchicine treatment (1). Another standpoint is that continuous axoplasmic-flow inhibition could lead, at the synaptic end, to a deficit in some proteins necessary for learning-related modification of synaptic efficacy (7). Colchicine treatment should then be without effect on retention, as in protein synthesis inhibitor studies. Finally, the learning impairment could also result from loss of cell shape control. This last hypothesis would predict a disruption by colchicine of both acquisition and maintenance of the behavior. We therefore analyzed the reversibility of chronic colchicine effect upon learning, as well as chronic colchicine effect upon retention of a well-acquired behavior. Saline- (n = 9) or colchicine- (60 μg/kg IP; n = 10) injected rats were submitted to the same operant procedure as previous experiments. After an initial 10-day learning period, the treatments were crossed for a further 5-day learning period, following a 2-day wash-out.

RESULTS

At the completion of the first 10-day learning period, colchicine induced a learning deficit similar to that of previous Experiments No. 1 and 3, as compared to saline controls [ANOVA (interaction drug × session): $F(9,153) = 19.66$, $p<0.0001$] (Fig. 2, left). However, after switching to saline, the rats previously given colchicine displayed the same learning rate as controls did in the first learning period [group colchicine-saline (session 11–15): slope (mean ± sd) = 15.99 ± 9.12 vs. group saline-colchicine (session 1–10): slope (mean ± sd) = 13.23 ± 3.07; $t(17df, 2\alpha) = 0.61$, p = NS] (Fig. 2, right). Conversely, trained control rats shifted to colchicine exhibited a progressive and significant drop in performance across sessions [group saline-colchicine (session 11–15): slope (mean ± sd) = −15.13 ± 7.34; $t(8df, 2\alpha) = 6.27$, $p<0.001$] (Fig. 2, right).

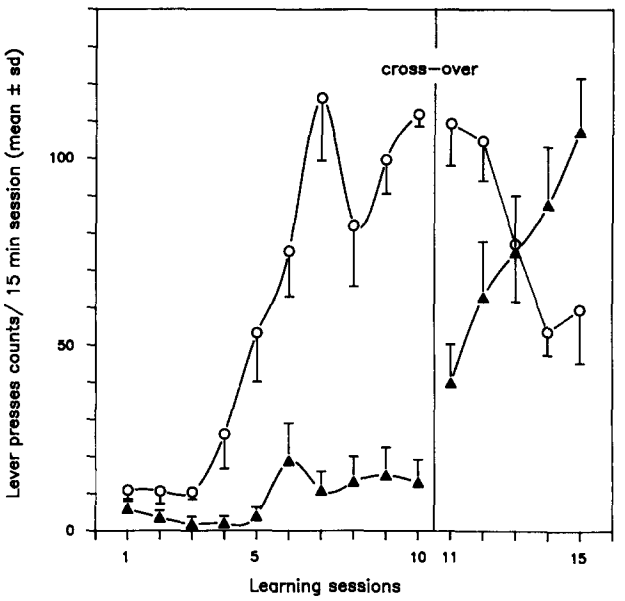

FIG. 2. Effect of chronic colchicine (60 μg/kg intraperitoneally 90 min before test) on a food-rewarded operant conditioning: lever press counts per 15-min session. Group 1 (n = 10) (▲) received colchicine (solid line) on session 1–10 (left) and was switched to saline (dashed line) on session 11–15 (right). Group 2 (n = 9) (○) received the treatments in reverse order. Session 1–10: group 1 (colchicine) demonstrates a significant deficit in acquisition compared to group 2 (saline), $F(9,153) = 19.66$, $p<0.0001$. Session 11–15: group 1, after switching to saline, displayed the same learning rates as group 2 in first period; group 2, after switching to colchicine, displayed a progressive response decrement.

Decreased drive toward food due to sickness, satiety or unrewarding effect of food could also result in such a response decrement pattern (35). Against these hypotheses, rats under colchicine treatment actually consumed each pellet obtained, as expressed by the very low difference between lever-press performance and pellet consumption, with no significant variation across sessions [group saline-colchicine (sessions 11–15): overall difference (mean ± sd) = 0.75 ± 1.34; ANOVA for repeated measures (session effect): $F(4,32) = 0.32$, p = NS]. Also the number of flap openings of the recess where pellets were delivered argues against an effect upon the perception of food as reward.

At the end of the 10 learning sessions, rats previously given saline displayed an excessive number of flap openings of the recess compared to the number of lever-presses [group saline-colchicine (session 10): nb visits (mean ± sd) = 155.7 ± 40.3 vs. nb lever-presses (mean ± sd) = 111.9 ± 9.9]. This number of non-rewarded flap openings can be considered as a measure of the strength of interest in food. When these well-trained control rats were shifted to colchicine, nonrewarded flap openings remained constant across sessions [group saline-colchicine (sessions 11–15): overall nonrewarded flap openings (mean ± sd) = 40.6 ± 34.9; ANOVA for repeated measures (session effect): $F(4,32) = 1.54$, p = NS]. Thus it can be concluded that the decrease in response was not related to the lower rewarding effect of food.

DISCUSSION

The present findings are, to our knowledge, the first to give evidence that chronic systemic colchicine administration in rats induces a deficit in a food-rewarded operant responding paradigm, both during and after acquisition. Amnesic effects of intracranial injections of colchicine are well established, but in most cases are explicitly mediated by cell lesioning and are limited to acquisition deficits (11, 23, 27–30). In earlier studies (2,6) amnesic effects of intracranial injections of colchicine were thought to be related to the blocking of newly synthetized protein axoplasmic transport. However, since in these studies colchicine doses were similar to, or sometimes higher than, the one used in lesioning studies, it seems very likely that a lesioning effect was already present. One other previous report alone made use of nonlesioning single systemic colchicine administration (60 μg/kg SC) on mice, but could not demonstrate any learning deficit (12). Though we used both different tasks and different animal species, which could account for our results, the major difference with the former study probably lays in the continuous disruption of microtubule function provided by the repeated administration procedures that we used. In pilot studies (unpublished data) we could not demonstrate a learning deficit, in mice or rats, using the same learning paradigm, specifically one-trial avoidance tasks, with single doses of colchicine ranging from 0.125 to 1 mg/kg IP.

That these effects are related to the microtubule blocking activity of colchicine is supported by the results in Experiment 3, showing that β-lumicolchicine at the same dose did not induce a learning deficit. Within the dose range studied, chronic colchicine induced neither motor incapacitation, nor decreased interest in food rewards, which could explain either deficit in acquisition or maintenance of the behavior. Thus these effects can be confidently interpreted in terms of deficit in memory processes. To what biological consequences resulting from continuous microtubule disruption these cognitive deficits are linked, is suggested by the results of Experiments 1 and 4. The deficit induced is dose-dependent, and is promptly and completely reversible following colchicine withdrawal. This, therefore, supports a transient pharmacological effect rather than a lesioning one. Since the continuous blocking of axoplasmic-flow would result in protein transport deficiency, the impairment of acquisition does not contradict the underlying assumptions in the protein synthesis inhibitor studies (7). However, the disruption of a well-established operant behavior, in Experiment 4, cannot support the hypothesis of an impairment mediated by axoplasmic transport inhibition. Short of histological indications to the contrary, the present results are compatible with our hypothesis of a mediation by the cell-morphology microtubule control of long-term memory formation and maintenance.

The animal model used was first designed to mimic, in the continuous disruption of microtubule function, a biological sequelae of Alzheimer's disease. The resulting memory impairments are quite relevant to cognitive defects in AD. Chronic colchicine induced a deficit in the acquisition of new learning and, foremost, unlike other pharmacological methods, it extended the range of cognitive impairment to the more resistant feature of memory, i.e., the expression of previously well-acquired behavior. Thus such an animal model of learning impairment and memory loss could help understand the pathogenesis of Alzheimer's disease and screen new drugs needed to relieve its cognitive disabilities.

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