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Psychopharmacological Profile of the Alkaloid Psychollatine as a 5HT2$_{A/C}$ Serotonin Modulator

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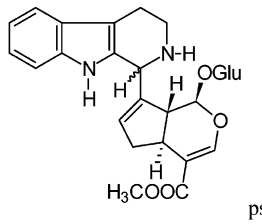
Behavioral effects of psychollatine, a new glycoside indole monoterpene alkaloid isolated from *Psychotria umbellata*, was investigated in models of anxiety, depression, memory, tremor, and sedation related to 5-HT and/or GABA neurotransmission. The GABA antagonist picrotoxin and the 5-HT2 antagonist ritanserin were used to examine the role of GABA and 5-HT2 receptors in psychollatine-induced effects. In the light/dark and hole-board models of anxiety, diazepam (0.75 mg/kg) and psychollatine (7.5 and 15 mg/kg) showed anxiolytic-like effect at doses that do not increase sleeping time nor alter spontaneous locomotor activity. The anxiolytic effect of psychollatine was prevented by prior administration of ritanserin, but not of picrotoxin, indicating that 5-HT2 but not GABA receptors are implicated. In the forced swimming model of depression, psychollatine (3 and 7.5 mg/kg) effects were comparable to the antidepressants imipramine (15 mg/kg) and fluoxetine (20 mg/kg). Psychollatine suppressed oxotremorine-induced tremors in all doses. In the step-down learning paradigm, diazepam (0.85 mg/kg), MK-801 (0.15 mg/kg), and psychollatine 100 mg/kg impaired the acquisition of learning and memory consolidation, without interfering with retrieval. It is concluded that the effects of psychollatine at the central nervous system involve serotonergic 5HT2$_{A/C}$ receptors.

Since its discovery over 50 years ago, serotonin (5-hydroxytryptamine, 5-HT) has been a long-standing target of intense research, in both academia and the pharmaceutical industry.[1] Current efforts focus on the identification of more potent and selective ligands for different receptor subtypes, aiming to enhance drug treatments with fewer side effects for a variety of disorders,[2] as well as understanding the diverse role of serotonin in the fine-tuning of various body functions. 5-HT shows a multitude of different physiological actions, and this is not surprising given the nature of the 5-HT neuronal system and the variety of different 5-HT receptors.[1] Currently, seven families of 5-HT receptors have been recognized.[2]

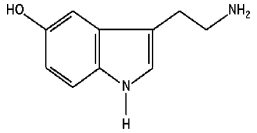
While early research on 5-HT was focused on its functions in peripheral tissues, a large proportion of current research exploit 5-HT pharmacology for therapeutic benefit associated with CNS functions.[1] 5-HT neurons originate in the hindbrain in a relatively circumscribed area, but send projections to most parts of the brain.[3] Furthermore, 5-HT is known to interact with other neurotransmitter systems, the literature being particularly rich regarding interactions between 5-HT and dopamine, and 5-HT and glutamate systems.[1,4]

The discovery of the selective 5-HT reuptake inhibitors represented an incremental step in the development of antidepressant drugs, and they are now the drugs of choice in treating depression.[1] Moreover, it has been long accepted that the regulation of fear and anxiety is strongly associated with the central γ-aminobutyric acid (GABA) and serotonergic (5-HT) systems.[5] A substantial amount of data has been accumulated on the role of various serotonin receptor subtypes in anxiety. Anxiolytic-like effects of drugs targeting serotonin 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ receptors have been revealed by conditioned procedures as well as ethological-based models.[6] Antagonism of the 5-HT$_3$ receptor also induces anxiolytic effects in various models of anxiety, and selective 5-HT$_3$ antagonists such as ondansetron and zacopride have anxiolytic profiles in rodents and potential clinical application in the treatment of generalized anxiety and panic disorders.[7,8] Recently, however, the significance of other neurotransmitter systems (such as cholinergic, dopaminergic, and glutamatergic) in modulating emotional behavior has received attention.[9]

Serotonergic projections arising from the raphe nuclei innervate limbic (amygdala and hippocampus) and cortical areas known to be involved with cognition and processing of emotional events.[10] 5-HT1 and 5-HT2 receptors are present in areas associated with learning and memory processes.[11,12] Although there is no consensus on the effects of enhancing or lessening serotonergic activity on mnemonic processes, differences in tasks and receptor subtypes appear to be relevant for any given outcome.[10−13]



psychollatine



5-hydroxytryptamine (serotonin)

Psychollatine (formerly known as umbellatine) is an indol-monoterpene alkaloid (isolated from *Psychotria um-*

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bellata Vell., Rubiaceae) structurally related to serotonin. The present study was undertaken to further investigate the psychopharmacological profile of psychollatine, by using the mouse light/dark and hole-board models, forced swimming test, step-down inhibitory avoidance, and oxotremorine-induced tremors. Locomotion, barbiturate sleeping time, and anticonvulsant activity (PTZ) were also examined. The role of 5-HT and GABA receptors in psychollatine mode of action were further evaluated.

Results and Discussion

In evolutionary terms, 5-HT is one of the oldest neurotransmitters and has been implicated in the etiology of numerous disease states.[2] Serotonin has been implicated in the regulation of nociception, motor behavior, endocrine secretions, cardiovascular function, and appetite, as well as in several psychiatric and neurological disorders.[14] Furthermore, 5-HT has been suggested to play a significant role in cognitive processes.[11,15] The 5-HT_{1A} and $5HT_{2A/C}$ receptor subtypes are especially relevant in this discussion, given its implication in both anxiety and depression[14,16] as well as with learning and memory processes.[12]

Diverse physiological and behavioral effects consequent to the stimulation of 5-HT_{1B} and 5-$HT_{2A/C}$ receptors have been reported;[11] for instance ritanserin, a nonselective 5-$HT_{2A/2C}$ receptor antagonist, was shown to be effective in improving several anxiety disorders, including panic and generalized anxiety.[17] Increased 5-HT activity in the brain (e.g., through agonists RU-24969 and *m*CPP) has been shown to induce spatial learning deficits, while decreased activity (e.g., antagonists ketanserin and ritanserin) improve consolidation of spatial discrimination, suggesting that drugs that stimulate or block the 5-HT_{1B} and/or 5-$HT_{2A/2C}$ receptors impaired or enhanced spatial learning, respectively.[11] Nevertheless, ritanserin and mianserin were prejudicial in conditioned eye blink in rabbits,[18] while ritanserin impaired elevated T-maze inhibitory avoidance in rats,[19] suggesting differential roles of serotonin receptors in diverse learning paradigms.

Hole-Board. The hole-board model is used to identify and evaluate the anxiolytic/anxiogenic properties of drugs.[20] As expected diazepam (0.75 mg/kg) increased the number of head-dips ($F_{(2,50)} = 51.14$; $P \leq 0.01$), crossings ($F_{(2,50)} = 89.93$; $P \leq 0.01$), and rearings ($F_{(2,50)} = 10.91$; $P \leq 0.01$) (Figure 2a−c, respectively). Psychollatine (7.5 and 15 mg/kg) also increased the number of head-dips ($F_{(5,85)} = 30.73$; $P \leq 0.01$), crossings ($F_{(5,85)} = 17.57$; $P \leq 0.01$), and rearings ($F_{(5,85)} = 12.99$; $P \leq 0.05$) (Figure 1a−c, respectively). The highest psychollatine dose (200 mg/kg) significantly reduced the number of head-dips and crossings ($P \leq 0.01$) (Figure 1a,b). None of the treated groups significantly differed from controls regarding latency to the first head-dip (data not shown). Pretreatment with picrotoxin reversed the effects of diazepam (Figure 2a−c) but not those of psychollatine in this model; pretreatment with ritanserin reversed the effects of psychollatine on head-dips ($F_{(2,27)} = 20.52$; $P \leq 0.01$), crossings ($F_{(2,27)} = 8.12$; $P \leq 0.01$), and rearings ($F_{(2,27)} = 6.27$; $P \leq 0.01$) (Figure 2a−c).

Results from hole-board show that diazepam and psychollatine have a clear and consistent effect (increased number) on head-dipping behavior (the key parameter) at doses that did not produce sedation. The effects of diazepam were prevented by pretreatment with the GABA antagonist picrotoxin, whereas the effects of psychollatine were antagonized by the $5HT_{2A/C}$ antagonist ritanserin.

Light/Dark Model. The light/dark model is a widely accepted animal model of anxiety.[21] Diazepam (0.85 mg/kg) significantly lengthened ($F_{(2,60)} = 12.14$, $P \leq 0.01$) the time spent in the lighted compartment, as well as the latency ($F_{(2,60)} = 16.86$, $P \leq 0.01$) to the first entry in the dark compartment (Figure 3a,b). Likewise, psychollatine (7.5 mg/kg) increased the time ($F_{(5,114)} = 6.51$, $P \leq 0.05$) spent in the light area and the latency ($F_{(5,114)} = 11.64$, $P \leq 0.01$) to the first entry in the dark compartment (Figure 3a,b). No significant differences were seen in the number of transitions between compartments among the various groups (data not shown). Pretreatment with picrotoxin reversed the effects of diazepam, but not those of psychollatine (Figure 4a,b). Pretreatment with ritanserin reversed the effects of psychollatine on the time spent in the light area ($F_{(2,22)} = 7.45$; $P \leq 0.01$) and the latency to the first entry in the dark compartment ($F_{(2,22)} = 2.32$, $P \leq 0.05$) (Figure 4a,b).

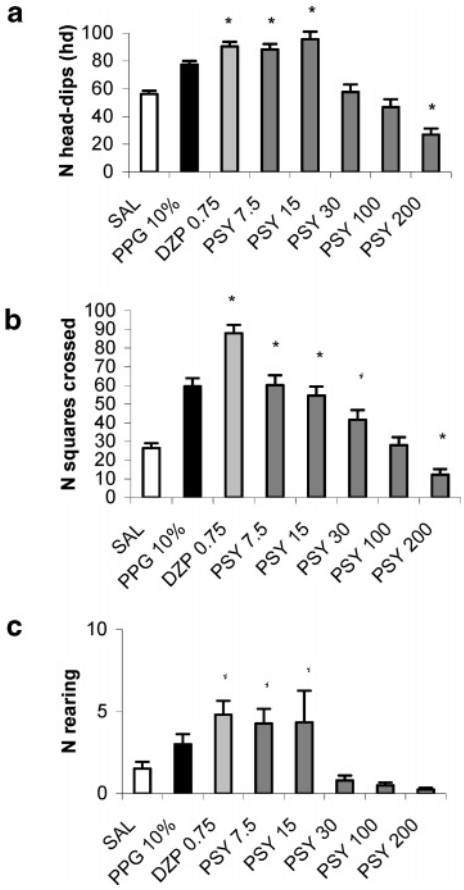

Figure 1. Effects of psychollatine in the hole-board test. Number of head-dips (a), number of squares crosses (b), and number of rearings (c). SAL; PPG 10%; DZP 0.75, 0.75 mg/kg; PSY 7.5, 15, 30, 100, and 200, psychollatine 7.5, 15, 30, 100, and 200 mg/kg. Each column represents the mean ± SEM ($N = 15$). ANOVA * = $P < 0.05$ and ** = $P < 0.01$ compared with controls.

In the light/dark model, results were consistent with those obtained with hole-board. Diazepam and psychollatine behaved as anxiolytics, markedly increasing the time spent in the lit box. Again, the effects of diazepam were prevented by pretreatment with the GABA antagonist picrotoxin, whereas the effects of psychollatine were antagonized by the $5HT_{2A/C}$ antagonist ritanserin. These data suggest that 5-$HT_{2A/C}$ receptors are involved in the anxiolytic properties of psychollatine. This result is consistent with the fact that psychollatine and serotonin are structurally related.

Potentiation of Barbiturate Sleeping Time and Spontaneous Locomotor Activity. To distinguish anxiolytic activity from general sedation, the following experi-

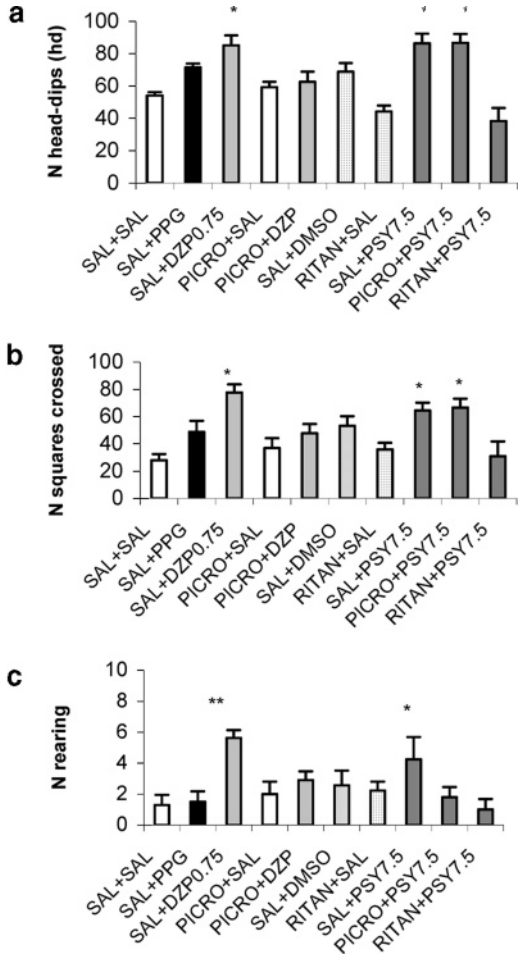

Figure 2. Effects of psychollatine and antagonists (picrotoxin and ritanserin) in the hole-board test. Number of head-dips (a), number of squares crosses (b), and number of rearings (c). SAL; PPG 10%; DMSO; DZP 0.75, 0.75 mg/kg; PSY 7.5, psychollatine 7.5 mg/kg; PICRO, picrotoxin 1 mg/kg; RITAN, ritanserin 2 mg/kg. Each column represents the mean ± SEM ($N = 8-14$). ANOVA * = $P < 0.05$ and ** = $P < 0.01$ compared with controls.

ments were conducted. Psychollatine (100 mg/kg) ($F_{(5,59)} = 6{,}55$, $P \leq 0.01$) and diazepam (2 mg/kg) ($F_{(2,29)} = 45.19$, $P \leq 0.01$) increased pentobarbital-induced sleeping time (Figure 5). As can be seen in Figure 6, saline, psychollatine (3, 7.5, 10, and 30 mg/kg), and diazepam (0.75 and 0.85 mg/kg) did not interfere with the spontaneous locomotion, whereas psychollatine (100 mg/kg) reduced locomotion [$F_{(5,78)} = 10.427$, $P < 0.01$].

Higher doses of psychollatine induces sedative effects, including potentiation of barbiturate sleeping time (100 mg/kg), marked inhibition of spontaneous ambulation (100 mg/kg), and deficits in rota-rod performance (200 mg/kg).[22] Sedative doses are distinctly separated from the anxiolytic dose range (7.5−15 mg/kg).

PTZ-Induced Convulsions. As can be seen in Figure 7, 100% and 90% of control animals (saline and PPG, respectively) presented seizures within 60 min after PTZ treatment. Both diazepam (0.8 mg/kg) (10%, $P \leq 0.05$) and phenobarbital (20 mg/kg) (0%, $P \leq 0.05$) protected mice from PTZ convulsions, whereas psychollatine was devoid of effect.

The fact that psychollatine does not behave as anticonvulsant against pentylenetetrazole nor has its anxiolytic effects altered by pretreatment with picrotoxin suggests that GABA receptors do not play a significant role in the behavioral effects of psychollatine.

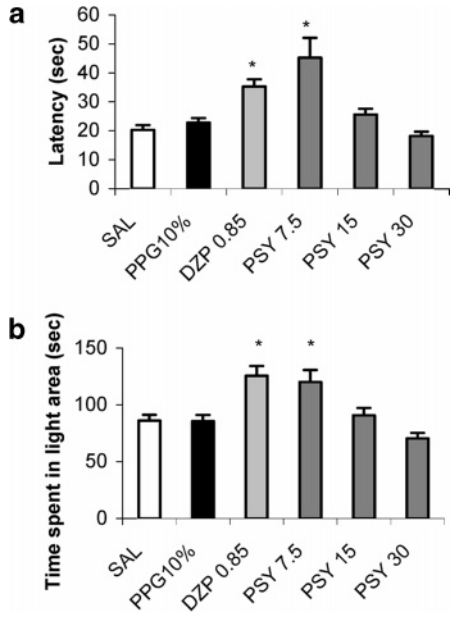

Figure 3. Effects of diazepam and psychollatine on the behavior of mice in the light/dark test. Latency of the first entry (a) and the amount of time spent by mice in the light area (b). SAL; PPG 10%; DZP 0.85, 0.85 mg/kg; PSY 7.5, 15, and 30; psychollatine 7.5, 15, and 30 mg/kg. Each column represents the mean ± SEM ($N = 19-22$). ANOVA * = $P < 0.05$ and ** = $P < 0.01$ compared with controls.

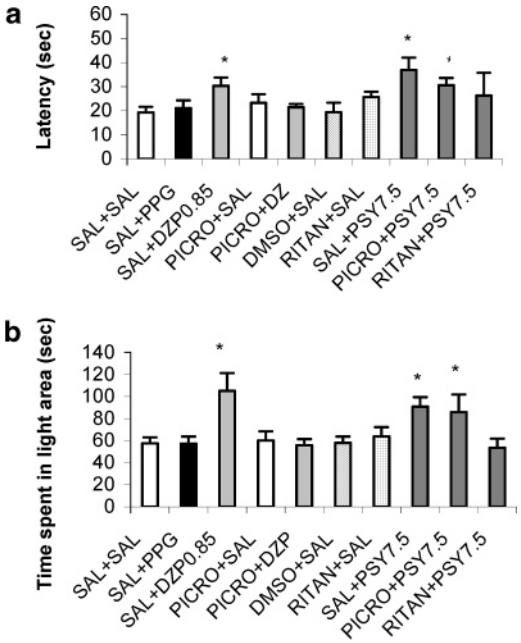

Figure 4. Effects of diazepam and psychollatine on the behavior of mice in the light/dark test with antagonists. Latency of the first entry (a) and the amount of time spent by mice in the light area (b). SAL; PPG 10%; DMSO; DZP 0.85, 0.85 mg/kg; PSY 7.5, psychollatine 7.5 mg/kg; PICRO, picrotoxin 1 mg/kg; RITAN, ritanserin 2 mg/kg. Each column represents the mean ± SEM ($N = 8-10$). ANOVA * = $P < 0.05$ and ** = $P < 0.01$ compared with controls.

As discussed above, in addition to anxiety, the serotonergic system has been implicated in the modulation of depression[14,16] and cognition.[11,12,15] Consequently, psychollatine effects were evaluated in the following relevant models.

Forced Swimming Test. Psychollatine at doses of 3 (48.88 ± 12.73 s) and 7.5 mg/kg (56.41 ± 7.22 s) significantly ($P < 0.05$) decreased the duration of immobility when compared with saline (112.53 ± 6.73 s). Higher doses (10 and 30 mg/kg) of psychollatine were devoid of effect.

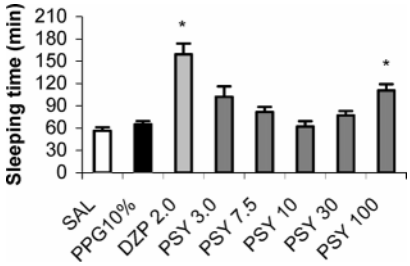

Figure 5. Effects of benzodiazepine (diazepam), psychollatine, and respective vehicle (PPG and saline) on potentiation of barbiturate sleeping time. SAL; PPG 10%; DZP 2.0, 2 mg/kg; PSY 3.0, 7.5, 10, 30, and 100, psychollatine 3, 7.5, 10, 30, and 100 mg/kg. Each column represents the mean ± SEM ($N = 9-12$). ANOVA * = $P < 0.05$ and ** = $P < 0.01$ compared with controls.

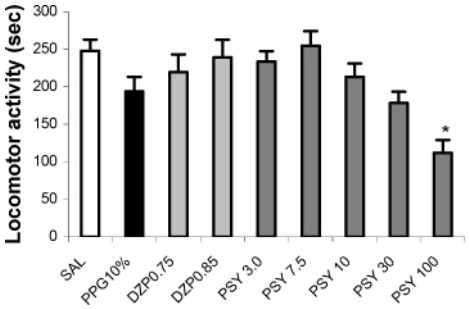

Figure 6. Effects of benzodiazepine (diazepam), psychollatine, and respective vehicle (PPG and saline) on spontaneous locomotor activity. SAL; PPG 10%; DZP 0.75 and 0.85, 0.75, and 0.85 mg/kg; PSY 3.0, 7.5, 10, 30, and 100, psychollatine 3, 7.5, 10, 30, and 100 mg/kg. Each column represents the mean ± SEM ($N = 9-12$). ANOVA * = $P < 0.05$ and ** = $P < 0.01$ compared with controls.

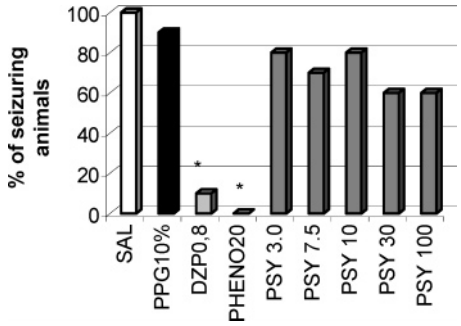

Figure 7. Effects of diazepam, phenobarbital, and psychollatine on PTZ-induced convulsions. SAL, PPG 10%; DZP 0.8, 0.8 mg/kg; PHENO 20, phenobarbital 20 mg/kg, PSY 3.0, 7.5, 10, 30, and 100, psychollatine 3, 7.5, 10, 30, and 100 mg/kg. N = 10. Fischer * = $P < 0.05$ and ** = $P < 0.01$ compared with controls.

MK-801 (0.15 mg/kg) (33.36 ± 3.94 s), imipramine (15 mg/kg) (46.05 ± 9.43 s), and fluoxetine (20 mg/kg) (57.61 ± 10.88 s) also reduced ($P < 0.05$) the duration of immobility (Figure 8).

Psychollatine (at doses that do not affect locomotion), as well as fluoxetine and imipramine, significantly reduced the total duration of immobility in the forced swimming test in mice, commonly used to predict clinical efficacy of several antidepressants.[23]

Oxotremorine-Induced Tremors. Oxotremorine is a potent muscarinic agonist, and its tremorigenic activity seems to be primarily mediated through central cholinergic stimulation.[24] Psychollatine at 3 mg/kg (7 [6−9]), 7.5 mg/kg (7 [6−9]), 10 mg/kg (6.5 [2−9.25]), 30 mg/kg (5.5 [3.75−7]), and 100 mg/kg (8[5.75−9]) and scopolamine at 3 mg/kg (0 [0−0]) suppress tremors in oxotremorine-treated mice when compared with saline (10 [9−11.5]). The effect of psychollatine was not dose-dependent (Figure 9).

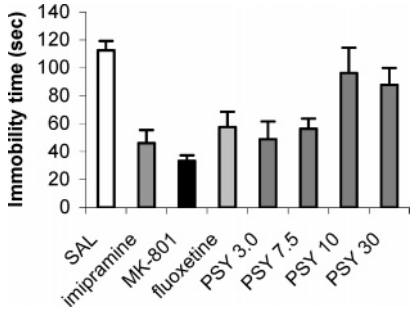

Figure 8. Effects of imipramine, fluoxetine, MK-801 and psychollatine on time of immobility in forced swimming test. PSY 3.0, 7.5, 10, and 30 = psychollatine 3, 7.5, 10, and 30 mg/kg. Each column represents the mean ± SEM ($N = 10$). ANOVA* = $P < 0.05$ compared with saline.

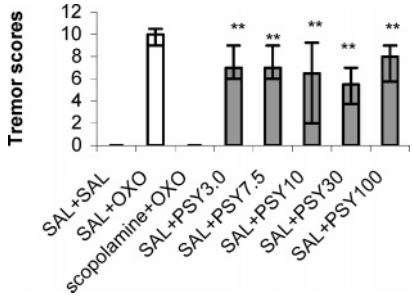

Figure 9. Effects of scopolamine and psychollatine on oxotremorine-induced tremor. Scopolamine 3 mg/kg; OXO, oxotremorine 0.5 mg/kg; PSY 3.0, 7.5, 10, 30, and 100, psychollatine 3, 7.5, 10, 30, and 100 mg/kg. Each column represents the median (interquartile ranges) of training (light columns) or test (dark columns) session latencies ** $P < 0.01$ significant difference compared with saline + oxotremorine in Mann−Whitney U test, following Kruskal−Wallis.

Step-Down Inhibitory Avoidance. Short-Term Memory (STM). Diazepam (0.85 mg/kg) (9.65 s[3.05−31.55]), MK-801 (0.15 mg/kg) (8.4 s[2.85−14.32]), and only the higher psychollatine dose (100 mg/kg) (6.8 s[3.85−17]) significantly reduced ($P < 0.01$) the latency during aquisition testing when compared with saline (29.2 s[8.4−137]) (Figure 10a). When tested for effects in memory retention, diazepam (0.85 mg/kg) (8.3 s[7.2−15.5]), MK-801 (0.15 mg/kg) (16.5 s[8.2−22.6]), and psychollatine at 3 mg/kg (11.8 s[5−21.9]), 7.5 mg/kg (9.6 s[4.45−19.37]), 10 mg/kg (11.55 s[4.87−19.92], and 30 mg/kg (12.8 s[4.47−34.17]) also caused a significant ($P < 0.01$) reduction in step-down latency when compared with saline (21.4 s[12.3−80]) (Figure 10b). No one treatment induced differences in retrieval (data not shown).

Long-Term Memory (LTM). Diazepam (0.85 mg/kg) (4.75 s[3−16.32]), MK-801 (0.15 mg/kg) (6 s[2.42−9.95]), and psychollatine at 7.5 mg/kg (13.4 s[5.12−25.72] and 100 mg/kg (5 s[2.3−17.57]) significantly reduced ($P < 0.01$) the latency during acquisition testing when compared with saline (33.2 s[13−114.6]) (Figure 10a). When tested for effects in memory retention, diazepam (0.85 mg/kg) (5.4 s[3−9.10]), MK-801 (0.15 mg/kg) (6.2 s[3.1−12.70]), and psychollatine at 7.5 mg/kg (13.1 s[3.02−90.325]) and 100 mg/kg (9.5 s[3.225−100.175]) also caused a significant ($P < 0.01$) reduction in step-down latency when compared with saline (50.3 s[18.20−156.3]) (Figure 10b). No differences in retrieval were found after treatments (data not shown).

It has been suggested that cross-talk between central serotonergic and cholinergic systems plays a critical role in mnemonic processes.[13] The hippocampal cholinergic tone may be modulated via 5-HT_{1A} receptor, the serotonin 5-$HT1_A$ agonist 8-OH-DPAT stimulates the release of acetylcholine in the hippocampus,[25] and fluoxetine reverses

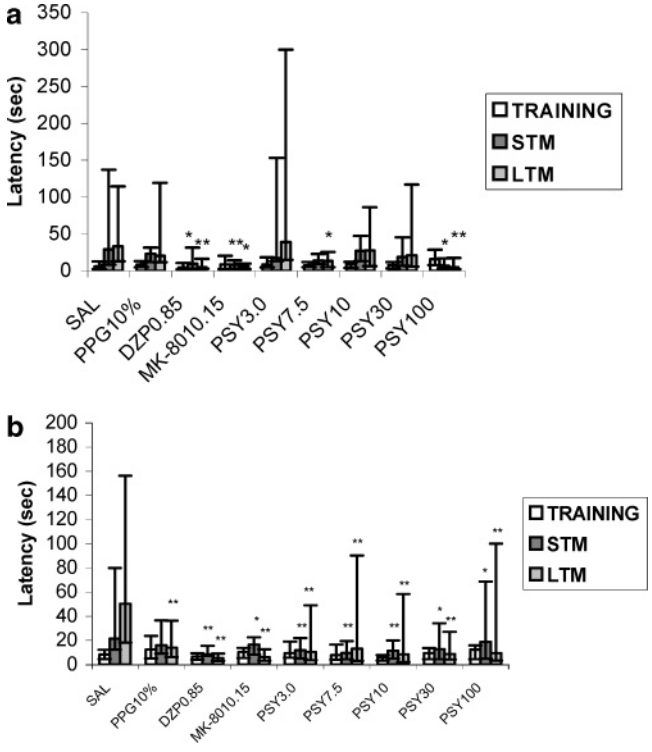

Figure 10. Effect of psychollatine given 30 min prior to training (A) and immediately posttraining (B) on test performance of adult mice trained in step-down inhibitory avoidance (0.3 mA footshock, 24 h training-test interval). $N = 20$ per group. Each column represents the median (interquartile ranges) of training (light columns) or test (dark columns) session latencies, for long-term memory (LTM) and short-term memory (STM). * $P < 0.05$, ** $P < 0.01$ significant difference compared with controls in Mann−Whitney U test, following Kruskal−Wallis.

scopolamine-induced cognitive deficit.[11] Several reports have indicated that tryciclic antidepressants, including clomipramine and imipramine, as well as selective serotonin reuptake inhibitors, such as paroxetine, inhibit oxotremorine-induced tremor,[26] and this anticholinergic activity may be responsible for the antidepressants' unwanted side effects in memory.[27] The overall effect of agents that primarily interfere with serotonergic receptors on the cholinergic system is, therefore, intricate. In the case of psychollatine, its effects on oxotremorine-induced tremors and memory suggest an overall depression of cholinergic transmission. The possibility that psychollatine-induced amnesia is the result of its modulation of serotonin per se cannot be ruled out at this point.

It has been accepted that changes in the serotonergic transmission can interfere with learning acquisition and memory consolidation; in fact, the brain structures implicated in learning and memory processes contain a large number of 5-HT receptors, as well as other neurotransmission systems.[28] Tryciclic antidepressants such as imipramine and amitryptiline, but not the selective serotonin reuptake inhibitor fluoxetine, have been reported to induce amnesia in passive avoidance and maze performance in mice.[26]

In this study psychollatine impaired acquisition and consolidation memory processes in the step-down inhibitory avoidance, without affecting memory retrieval. This pattern is compatible with those obtained with 5-HT agonists[11] and antagonists,[19] muscarinic antagonists,[29] and NMDA glutamate antagonists.[30] Our current data suggest that psychollatine interferes with serotonergic transmission and possibly with the cholinergic systems. Considering the previously reported analgesic effect of psychollatine in the capsaicin model, and its synergism with MK-801, a potential role of NMDA receptors in psychollatine amnesic effect has to be taken into account.[22]

Complete pictures of the molecular mechanism underlying the effects of psychollatine as anxiolytic, antidepressive, and amnesic remain to be elucidated. However, this study adds to the idea that the indole monoterpene alkaloid psychollatine deserves further investigation as a useful template to develop selective subtype ligands for 5-HT receptors.

Experimental Section

General Experimental Procedure. Diazepam (DZP), sodium pentobarbital, ritanserin, picrotoxin, phenobarbital, and propylene glycol (PPG) were acquired from Sigma; DMSO from Delaware; pentylenetetrazol (PTZ) from Knoll A.G-Ludwingshafen/Rheno; and MK-801 from RBI. Drugs and vehicles were administered intraperitoneally (i.p.), except for PTZ, given subcutaneously (s.c.), always as 0.1 mL/10 g of body weight. Diazepam (0.75, 0.85, and 2 mg/kg) was suspended in propylene glycol 10% (v/v). Ritanserin (2.0 mg/kg) was suspended in DMSO 10% (v/v). Psychollatine (3, 7.5, 10, 30, 100, and 200 mg/kg) was solubilized in one or a few drops (20−60 μL) of HCl (1 N), the final volume adjusted with saline and the pH adjusted (7.0) with a few drops of NaOH (1 N). Sodium pentobarbital (40 mg/kg), PTZ (88 mg/kg), phenobarbital (20 mg/kg), picrotoxin (1.0 mg/kg), MK-801 (0.15 and 0.25 mg/kg), scopolamine (3 mg/kg), and oxotremorine (0.5 mg/kg) were diluted in saline. Control groups received saline (NaCl 0.9%), PPG (10%), or DMSO (10%) as appropriate.

Plant Material. *Psychotria umbellata* Vell. (Rubiaceae) leaves were collected and identified by Gert Hatchbach, in February 1995; a voucher (MBM 48571) has been deposited at the herbarium of the Museu Botânico Municipal de Curitiba (PR, Brazil).

Extraction and Isolation. Dried leaves (100 g) were extracted with EtOH at room temperature three times, each for a week. The extract was concentrated under vacuum at 40 °C to a dark green syrup. The syrup was dissolved in 2% HCl (0.5 L) and partitioned with CH_2Cl_2. The acid solution was alkalinized with 25% ammonia solution until pH = 10 and extracted with CH_2Cl_2. From the CH_2Cl_2 extract 954 mg of a colorless amorphous compound was precipitated. Purity of the compound was checked by TLC with silica gel 60F254 ($CHCl_3$/MeOH-NH_3 vapor, 85:15; R_f = 0.2) and HPLC (column: NOVAPACK C18 150 mm × 3.9 mm, Waters; MeOH/H_2O, 50:50, as eluent and a photodiode array as detector; t_R = 2.13 min).

Animals. Experiments were performed with male adult mice (CF1), acquired from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS) at 2 months of age. Animals were maintained in our own facilities (22 ± 1 °C, 12 h light/dark cycle, free access to food [Nuvilab CR1] and water) for at least two weeks before experiments. All procedures were carried out according to institutional policies on experimental animal handling.

Hole-Board Model. The hole-board apparatus (Ugo Basile, Italy) consisted of a gray Perspex panel (40 × 40 × 40 cm, 2.2 cm thick) with 16 equidistant holes (3 cm in diameter) in the floor. Photocells below the surface of the holes provided measures of the number of head-dips. The board was positioned 15 cm above the table and divided (with black water-resistant marker) in 16 squares of 10 × 10 cm. The method was adapted from Takeda et al. (1998).[20] Mice were transported to the dimly lit laboratory at least 1 h prior to testing. The animals were divided into 10 groups (N = 15) and treatments (saline, PPG, DMSO, diazepam, and psychollatine) administered 30 min prior to the testing. After 30 min each animal was individually placed in the center of the board (facing away from the observer) and the following parameters were noted for 5 min: the latency to the first head-dip measured using a stopwatch; number of head-dips; the number

of rearings and spontaneous movements (number of squares crossed with all four paws). To verify the influence of the different receptors, the antagonists ritanserin and picrotoxin were administered 30 min before psychollatine (7.5 mg/kg). Statistical analysis involved an initial one-way analysis of variance (ANOVA), followed by a Student Newman Keuls (SNK) test.

Light/Dark Model. This model of anxiety was based on that described by Misslin et al. (1989)[31] and consists of an open-topped rectangular box (48 × 29 × 46 cm high), divided into a small (19 × 29 cm) and a large (29 × 29 cm) area. The mice could move from one box to the other through an open door (7 × 7 cm) between the two boxes. The small compartment was painted black and provided only with room illumination, whereas the larger compartment was painted white and brightly illuminated with a 60 W (400 lx) light source. Mice were transported to the experimental room, lit by a single dim red light, at least 1 h prior to testing. The animals were divided into 10 groups (N = 8−15) and treatments (saline, PPG, DMSO, diazepam, and psychollatine) administered 30 min prior to testing. After 30 min each animal was individually placed in the center of the illuminated box, facing the entrance to the dark box, and the following parameters were noted for 5 min: the latency of the first crossing, the amount of time spent in the light area, and total number of crossings from one compartment to the other. To verify the influence of the different receptors, ritanserin and picrotoxin were administered 30 min before psychollatine (7.5 mg/kg). Statistical analysis involved an initial one-way analysis of variance (ANOVA), followed by a Student Newman Keuls (SNK) test.

PTZ-Induced Convulsions. Mice were divided in five groups (n = 10), and test drugs (saline, PPG, diazepam, phenobarbital, and psychollatine) were given intraperitoneally. After 30 min mice received PTZ (88 mg/kg, subcutaneously). Following PTZ injection, animals were individually placed in transparent acrylic chambers (20 × 20 × 20 cm) and observed during 60 min for the presence of clonic convulsions lasting more than 3 s.[32] Data were analyzed through the Fisher's exact test.

Potentiation of Barbiturate Sleeping Time. Thirty minutes after treatment with saline, PPG, diazepam, and psychollatine (3−100 mg/kg) groups of eight mice (N = 9−12) were treated i.p. with 40 mg/kg of sodium pentobarbital. The sleeping time (time elapsed between loss and recuperation of righting reflex) was recorded. The adopted criterion for recuperation of righting reflex was that animals have to regain their normal posture for three consecutive times when challenged to remain on their backs.[33] Statistical analysis involved an initial one-way analysis of variance (ANOVA), followed by the Student Newman Keuls (SNK) test.

Spontaneous Locomotor Activity. The method was adapted from Creese et al. (1976).[34] Activity cages (Albarsch Electronic Equipment, 45 × 25 × 20 cm) equipped with three parallel photocells automatically record the number of crossings. Animals were individually habituated to an activity cage for 10 min before receiving the following treatments (n = 10−16): saline, psychollatine (3−100 mg/kg), and diazepam (0.75 and 0.85 mg/kg). Thirty minutes after treatments the animals returned to the activity cages, and crossings were recorded for 15 min. Statistical analysis involved an initial one-way analysis of variance (ANOVA), followed by the Student Newman Keuls (SNK).

Forced Swimming Test. We followed the method of Porsolt et al. (1977)[23] as modified by Sunal et al. (1994).[35] Mice were individually forced to swim in an open cylindrical container (diameter 30 cm, height 25 cm), containing 20 cm of water at 25 ± 1 °C, for 6 min. The duration of immobility was recorded during the last 4 min of the 6 min testing period. After vigorous activity, swimming attempts cease and the animal adopts a characteristic immobile posture. A mouse is judged to be immobile when it floats in an upright position and makes only small movements to keep its head above water. At the end of the session mice were removed and dried with a towel. Statistical analysis involved an initial one-way analysis of variance (ANOVA), followed by the Student Newman Keuls (SNK) test.

Oxotremorine-Induced Tremors. Saline, scopolamine, and psychollatine (3, 7.5, 10, 30, and 100 mg/kg) were administered to mice 30 min before an oxotremorine (0.5 mg/kg) injection. For observation, mice were individually placed in transparent acrylic cages (20 × 20 × 20 cm) immediately after oxotremorine administration; tremors was scored visually at 5 min intervals for 30 min using the following rating scale: 0 = no tremors, 1 = intermittent moderate tremors, and 2 = continuous severe tremors. Data are expressed as the total sum scores obtained in 30 min[36] and are expressed as median (interquartile ranges). Data were analyzed by Kruskal−Wallis nonparametric analysis of variance; comparisons between groups were run using the Mann−Whitney U-test (two-tailed).

Step-Down Inhibitory Avoidance. The test used was adapted from Netto and Izquierdo (1985)[37] and from Maurice et al. (1994).[29] Mice were habituated in the dim lighted room for at least 30 min before the experiments. The inhibitory avoidance training apparatus was a plastic box of 30 × 30 × 40 cm, with a platform (5 × 5 × 4 cm) fixed in the center of the grid floor. Each mouse was placed on the platform, and the latency to step down (four paws on the grid) was automatically recorded in training and test sessions. In the training session, the mouse received a 0.3 mA scrambled foot shock for 15 s upon stepping down. Animals exhibiting step-down latencies greater than 30 s in training were excluded from experiments; less than 5% of the animals met this exclusion criterion. The test session was performed 24 h later, with the same procedure except that no shock was administered after stepping down; an upper cutoff time of 300 s was set.

Drugs (saline, PPG 10%, MK-801, diazepam, or psychollatine) were administered as follows: 30 min before training, to evaluate effects on task acquisition (in this case no exclusion criteria were applied); immediately after training, to evaluate effects on memory consolidation; and 30 min before testing, to assess memory retrieval. The step-down latencies are expressed as median (interquartile ranges). Data were analyzed by Kruskal-Wallis nonparametric analysis of variance; comparisons between groups were run using the Mann−Whitney U-test (two-tailed). Comparisons between training and test sessions within each group were made by the Wilcoxon test. The Spearman-rank correlation coefficient was used to check dose−effect associations.

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References and Notes

(1) Jones, B.; Blackburn, T. *Pharmacol. Biocem. Behav*. **2002**, *71*, 555−568.
(2) Hoyer, D.; Hannon, J. P.; Martin, G. R. *Pharmacol. Biochem. Behav.* **2002**, *71*, 533−554.
(3) Azmitia, E. C.; Whitaker-Azmitia, P. M. *J. Clin. Psychiatry* **1991**, *52* (12), 4−16.
(4) Costa-Campos, L.; Dassoler, S. C.; Rigo, A. P.; Iwu, M.; Elisabetsky, E. *Pharmacol. Biochem. Behav.* **2004**, *77* (3), 481−489.
(5) File, S. E. *Neuropharmacology* **1987**, *26* (7B), 877−886.
(6) Bourin, M.; Dhonnchadha, B. A. N.; Colombel, M. C.; Hascoët, M. *Behav. Brain Res.* **2001**, *124*, 87−95.
(7) Kelley, S. P.; Bratt, A. M.; Hodge, C. W. *Eur. J. Pharmacol*. **2003**, *461*, 19−25.
(8) Freeman, A. M., III; Westphal, J. R.; Norris, G. T.; Roggero, B. A. *Depress. Anxiety* **1997**, *5*, 140−141.
(9) Nakamura, K.; Kurasawa, M. *Eur. J. Pharmacol.* **2001**, *420*, 33−43.
(10) Misane, I.; Ögren, S. O. *Neuropsychopharmacology* **2000**, *22* (2), 168−190.
(11) Meneses, A.; Hong, E. *Behav. Brain Res.* **1997**, *87*, 105−110.
(12) Khalifa, E. A. *Pharmacol. Biochem. Behav.* **2003**, *75*, 755−762.
(13) Solana-Figueroa, R.; Salado-Castillo, R.; Quirarte, L. G.; Galindo, E. L.; Prado-Alcalá, A. R. *Life Sci.* **2002**, *71*, 391−399.
(14) Beneytez, M. E.; López Rodríguez, M. L.; Rosado, M. L.; Morcillo, M. J.; et al. *Eur. J. Pharmacol.* **1998**, *344*, 127−135.
(15) Liang, K. C. *Eur. J. Neurosci*. **1999**, *11*, 1491−1500.
(16) Fletcher, A.; Forster, E. A.; Bill, D. J.; Brown, G.; et al. *Behav. Brain Res.* **1996**, *73*, 337−353.
(17) Celeumans, D. L.; Hoppenbrouwers, M. L.; Gelders, Y. G.; Reyntjens, A. J. *Pharmacopsychiatry* **1985**, *18*, 303−305.
(18) Harvey, J. A. *Learn. Mem.* **2003**, *10*, 355−362.

(19) Zangrossi, H., Jr.; Viana, M. B.; Zanoveli, J.; Bueno, C.; Nogueira, R. L.; Graeff, F. G. *Neurosci. Biobehav. Rev.* **2001**, *25*, 637−645.
(20) Takeda, H.; Tsuji, M.; Matsumiya, T. *Eur. J. Pharmacol.* **1998**, *350*, 21−29.
(21) Gard, P. R.; Haigh, S. J.; Cambursano, P. T.; Warrington, C. A. *Pharmacol. Biochem. Behav.* **2001**, *69*, 35−40.
(22) Both, F. L.; Kerber, V. A.; Henriques, A. T.; Elisabetsky, E. *Pharm. Biol.* **2002**, *40* (5), 336−341.
(23) Porsolt, R. D.; Bertin, A.; Jalfre, M. *Arquives Int. Pharmacodyn. Ther.* **1977**, *229*, 327−336.
(24) Fukuzaki, K.; Kamenosono, T.; Nagata, R. *Pharmacol. Biochem. Behav.* **2000**, *65* (3), 503−508.
(25) Lazaris, A.; Bertrand, F.; Lazarus, C.; Galani, R.; Stemmelin, J.; Poirier, R.; Kelche, C.; Cassel, J.-C. *Brain Res.* **2003**, *967*, 181−190.
(26) Fujishiro, J.; Imanishi, T.; Onozawa, K.; Tsushima, M. *Eur. J. Pharmacol.* **2002**, *454*, 183−188.
(27) Jain, N. N.; Ohal, C. C.; Shroff, S. K.; Bhutada, R. H.; Somani, R. S.; Kasture, V. S.; Kasture, S. B. *Pharmacol. Biochem. Behav*. **2003**, *75*, 529−536.
(28) Breese, R. G.; Knapp, J. D.; Moy, S. S. *Neurosci. Biobehav. Rev.* **2002**, *26*, 441−455.
(29) Maurice, T.; Hiramatsu, M.; Itoh, J.; Kameyama, T.; Hasegawa, T.; Nabeshima, T. *Brain Res.* **1994**, *647*, 44−56.
(30) Hlinák, Z.; Krejcí, I. *Behav. Brain Res.* **2002**, *131*, 221−225.
(31) Misslin, R.; Belzung, C.; Vogel, E. *Behav. Process.* **1989**, *8*, 119−132.
(32) Clark, C. R. *Epilepsia* **1988**, *29*, 198−203.
(33) Yamamoto, I.; Kimura, T.; Tateoka, Y.; Watanabe, K.; Kang Ho, I. *Life Sci.* **1987**, *41*, 2791−2797.
(34) Creese, I.; Burt, D. R.; Snyder, S. H. *Science* **1976**, *192*, 481−483.
(35) Sunal, R.; Gümüsel, B.; Kayaalp, S. O. *Pharmacol. Biochem. Behav.* **1994**, *49* (4), 891−896.
(36) Shuto, H.; Kataoka, Y.; Kanaya, A.; Matsunaga, K.; Sueyasu, M.; Oishi, R. *Eur. J. Pharmacol.* **1998**, *341*, 33−37.
(37) Netto, C. A.; Izquierdo, I. *Behav. Neural Biol*. **1985**, *43*, 327−330.

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