

EFFECTS OF (-)TETRAHYDROPALMATINE ON THE
ISOLATED ILEUM OF GUINEA-PIG.

Roberto L. Rojas, Concepción L. Martínez, José Anías

Instituto Superior de Ciencias Médicas de La Habana “Dr. Carlos J. Finlay”,
Calle 146 y Ave. 31, Playa, La Habana, CP 11600, Cuba

Email: rluis.rojas@infomed.sld.cu

Summary

Introduction: (-)tetrahydropalmatine (THP) is an isoquinoline alkaloid extracted from the *Stephania glabra* (Roxb) Miers and other species of plants. It produces sedation of the nervous system, which is attributable to its action on dopaminergic receptors localized in brain cortex and brain stem. **Objective:** To characterize the effects of THP as antagonist on different types of pharmacological receptors. **Methods:** The antagonisms produced by THP, chlorpromazine and papaverine vis à vis the contractions induced by acetylcholine, serotonin, histamine and bradykinin in the isolated guinea-pig ileum were compared. **Results:** THP inhibited the contractions induced by the four agonists in a non-specific similar way to papaverine, though the former was three times less active than the latter. However, chlorpromazine inhibited much more markedly the histamine and serotonin effects. **Conclusion:** These results were interpreted on the basis of the structural similarity of THP and papaverine. It is presumable that the collateral effects of THP will differ to those of chlorpromazine since its action spectrum on pharmacological receptors is unlike. This provides an incentive for the pharmacological evaluation of THP, with a view to its usage in human beings on account of its potential value as agent acting on the central nervous system with a low toxicity. **KEYWORDS:** tetrahydropalmatine, chlorpromazine, papaverine, gindarin, rotundine, pharmacological receptors, isolated ileum, guinea-pig

(-)tetrahydropalmatine (THP), also called gindarin or rotundine, is an isoquinoline alkaloid of the type of the tetrahydroprotoberberins. It produces sedation on the central nervous system, which can be attributed at least partially to its action on dopaminergic receptors localized in brain cortex and brain stem, and whose effects resemble those of a neuroleptic drug.(1) In that sense, it diminishes the motor activity in rats, while high dose produces rigidity, increases the effect of haloperidol, and reduces the hypermotility induced by apomorphine.(2)

Among its actions at the level of the central nervous system, it has been also reported a blockade of the motor effects and convulsions induced by picrotoxin(3), as well as those resulting of the electrical stimulation of these nervous structures.(4)

Some other effects observed with the administration of THP, namely arterial hypotension and bradycardia are also ascribed to inhibition of dopaminergic D2 receptors in central nervous system(5), but other actions are additionally involved, such as blockade of 5-HT2 serotonin receptors(6,7), alpha-1 and alpha-2 adrenergic receptors.(8,9) Antiarrhythmic action(9), blockade of calcium channels(10), analgesia(11) and hypothermia(12) are also reported.

Nevertheless the diversity of actions and effects described for THP, it is remarkable that its chemical structure resembles that of papaverine, which lacks specificity for any pharmacological receptor. Taking into account that the collateral effects of neuroleptics depend on actions over different kinds of central and peripheral receptors(13), it was somehow interesting to investigate if THP had “papaverine-like” action on pharmacological receptors, as a contribution to its characterization like an active principle.

Material and methods

Thirty male Dunkin-Hartley guinea pigs weighing 250-300 g were sacrificed to extract ileum segments following the usual procedure.(14) Isolated strips of ileum were introduced in a 10 mL glass chamber containing Tyrode solution under oxygen bubbling to a constant temperature of 37° C. Initial tension was 1 g. Sixty minutes later the experiments began. Contractions were registered through isotonic transducers coupled to a Nihon-Kohden polygraph. The agonist drugs used were acetylcholine, histamine, serotonin, bradykinin, and the antagonists were chlorpromazine, papaverine and (-) tetrahydropalmatine. The latter was obtained as hydrochloride from the Chemistry Laboratory of the University of Havana, where its preparation and isolation was informed.(15) Concentrations refer always to Moles/Liter.

Experimental design

Once the effector organ was stabilized, a submaximal contraction was induced with each agonist every five minutes allowing time enough to get the total effect of the added dose. Washing between additions was performed. After completing one cycle of four contractions with the agonists, one hour of incubation with the tested antagonist was achieved. Then a second cycle of contractions was carried out in the same order of agonists as the first one while the antagonist remained into the solution. The inhibition observed in the presence of the antagonist was expressed taking the size of the first corresponding contraction as 100 %. In successive experiments the order of agonist administration was changed to avoid tachyphylaxis; anyway, it was not observed. Only one concentration of antagonist drug was essayed with each strip. Incubation in control experiments was performed by adding to the glass chamber an analogous volume of Tyrode solution.

Statistics

One-way analysis of variance (ANOVA) was applied to the values of agonist inhibition for each dose of the antagonists. In case of significant difference ($p < 0.05$), then a Duncan’s multiple range test was applied to the involved values. Student’s test was used when only two means were contrasted.(16)

Results

Both papaverine and THP produced a dose-dependent inhibition of the four agonists used, and the extent of inhibition was approximately similar for all of them into the same concentration of the concerned antagonist. Accordingly, there was no significant difference among the values involved into the same antagonist concentration. (Fig. 1 and 2)

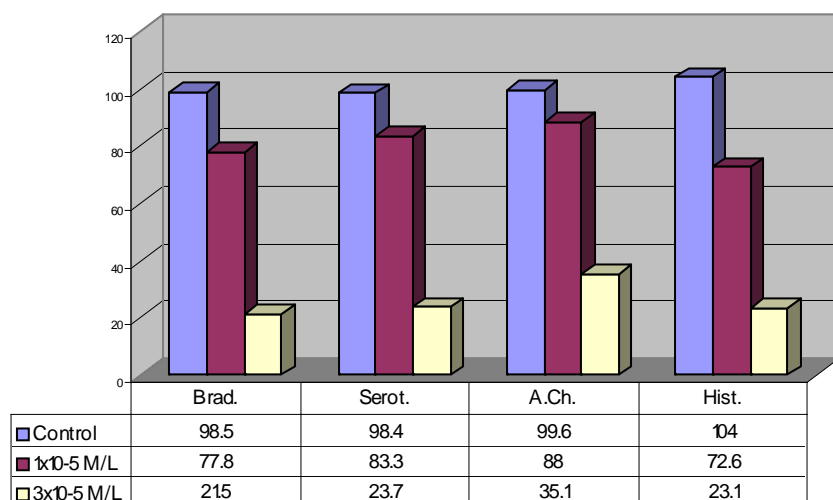


Fig. 1 Effect of papaverine on the contractions induced by the agonists. (N = 5 for each value. ANOVA: Control: df = 3,16, F = 0.38, $p > 0.5$; 1×10^{-5} M/L: df = 3,16, F = 1.18, $p > 0.3$; 3×10^{-5} M/L: df = 3,16, F = 0.54, $p > 0.5$)

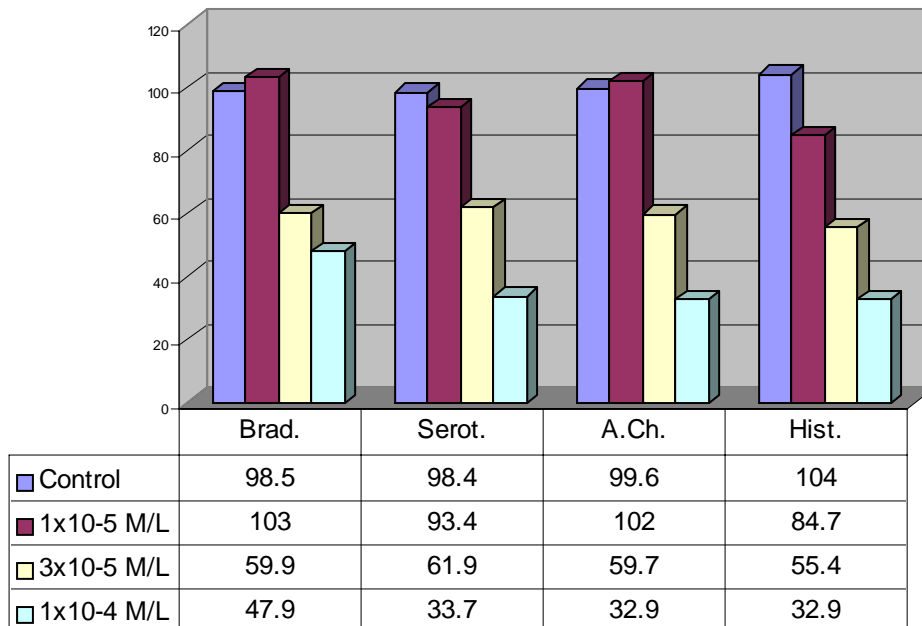


Fig. 2 Effect of THP on the contractions induced by the agonists. (N = 5 for each value. ANOVA: Control: df = 3,16, F = 0.38, p > 0.5; 1x10⁻⁵ M/L: df = 3,16, F = 2.13, p > 0.1; 3x10⁻⁵ M/L: df = 3,16, F = 0.04, p > 0.5; 1x10⁻⁴ M/L: df = 3,16, F = 0.41, p > 0.5)

Chlorpromazine antagonized completely the histamine-induced contractions at concentrations that did not affect those induced by bradykinin; there was a significant difference between them. On the other hand, the contractions of acetylcholine and serotonin were reduced in a dose-dependent manner, whose intensity was similar for the two drugs. At the concentration of 1 x 10⁻⁶ M/L, histamine and bradykinin differed significantly each one in relation to the other agonists, as well as serotonin and acetylcholine did, but there was no difference between them for the last two drugs. (Fig. 3)

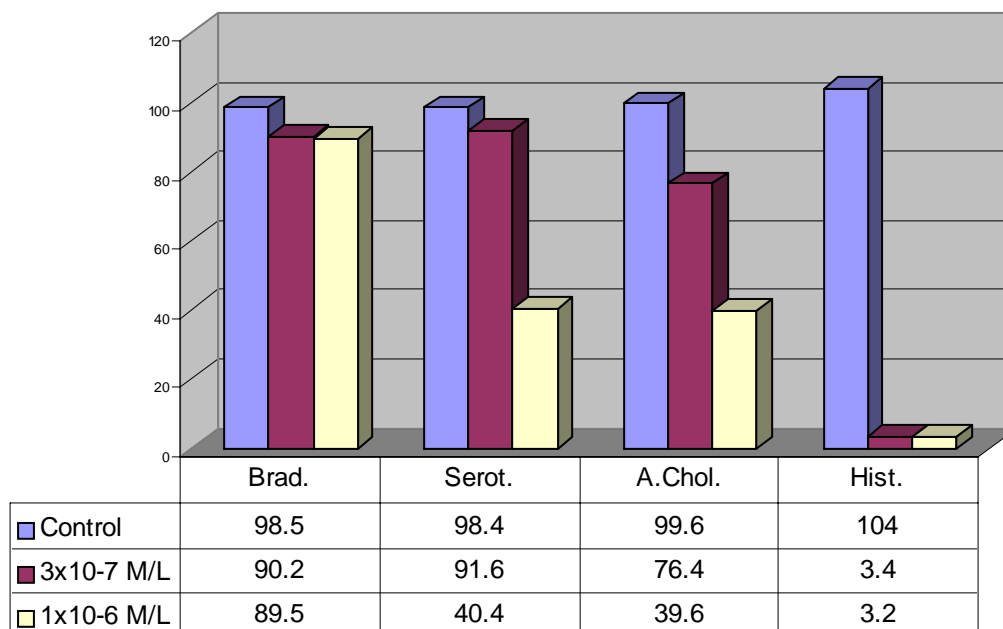


Fig. 3 Effect of chlorpromazine on the contractions induced by the agonists. (N = 5 for each value. ANOVA: Control: df = 3,16, F = 0.38, p > 0.5; 3x10⁻⁷ M/L: df = 3,16, F = 38.5, p < 0.001; 1x10⁻⁶ M/L: df = 3,16, F = 15.1, p < 0.001) (Duncan's test: 3x10⁻⁷ M/L: Histamine differs of all the others, p < 0.01; 1x10⁻⁶ M/L: Histamine and bradykinin differ of the others, p < 0.01; serotonin and acetylcholine do not differ between them, p > 0.05)

The mean effective concentration (EC50) in Mol/Liter of each antagonist facing each agonist is presented in table 1 (N = 5 for each EC50), as well as the mean value (± SE; N = 4) of the EC50 of papaverine and THP. T-test showed a very significant difference between THP and papaverine.

AGONIST	EC50 (M/L) OF ANTAGONIST		
	Papaverine	THP	Chlorpromazine
Acetylcholine	2.2×10^{-5}	5.1×10^{-5}	7.1×10^{-7}
Histamine	1.6×10^{-5}	4.4×10^{-5}	5.8×10^{-9}
Serotonin	1.9×10^{-5}	5.1×10^{-5}	8.0×10^{-7}
Bradykinin	1.7×10^{-5}	7.3×10^{-5}	No antagonism
Mean EC50 (\pm SE)	$1.8 (0.13) \times 10^{-5}$	$5.5 (0.63) \times 10^{-5}$	
	(Df = 6; t = 5.75; p < 0.005)		

Table 1. Value in Mol/Liter of the Mean Effective Concentration (EC50) of the antagonists facing the submaximal contractions induced by each agonist (N = 5 for each EC50). The mean value of EC50 (\pm SE; N = 4) of papaverine and THP, and the result of applying t-test are also presented.

Discussion

The neuroleptic drugs may act with a variable affinity on different types of pharmacological receptors. Frequently this diversity of actions becomes a restriction to their use because of the appearance of collateral effects. That is the case of chlorpromazine, a prototype drug for neuroleptics, which was employed in this study as a drug of reference.(13)

However, figures 1 and 2 show that papaverine and THP exhibited a qualitatively similar behavior, allowing to state that in our experimental conditions THP displayed a papaverine-like action in the intestinal smooth muscle of guinea-pigs. It means that THP did not have specificity by any of the pharmacological receptors studied in this effector organ.(17) This papaverine-like action may be understood on the basis of the resemblance between the chemical formula of both drugs. (Fig. 4, 5, 6)

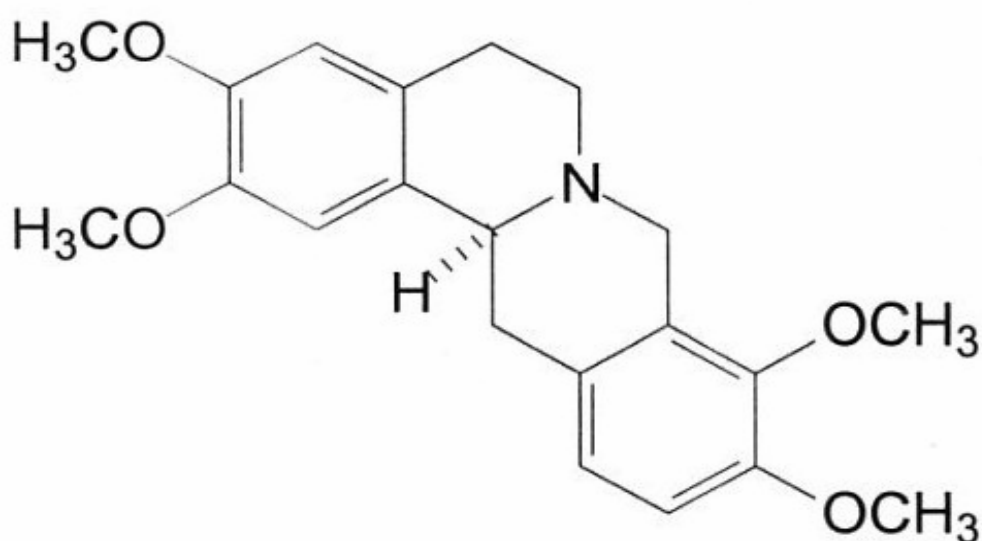


Fig. 4 Molecular structure of (1)tetrahydropalmatine.

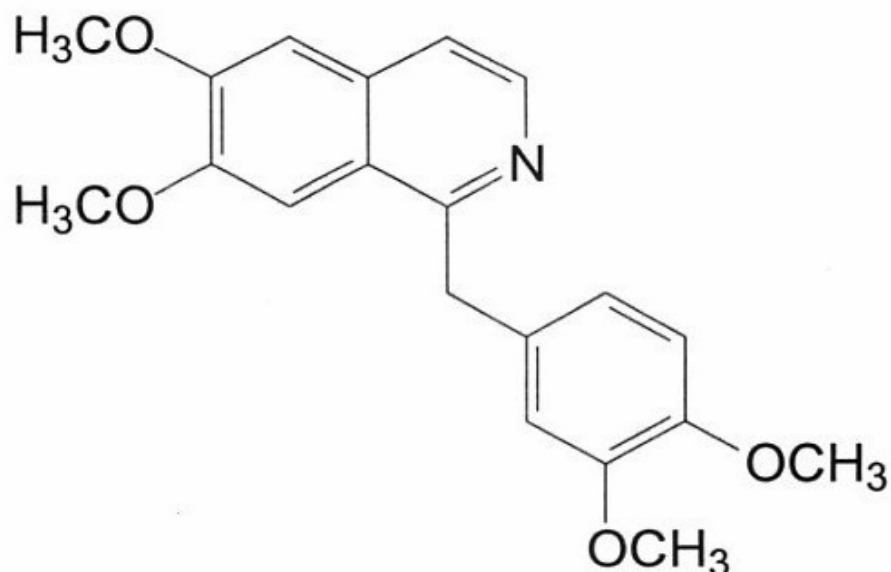


Fig. 5 Molecular structure of papaverine.

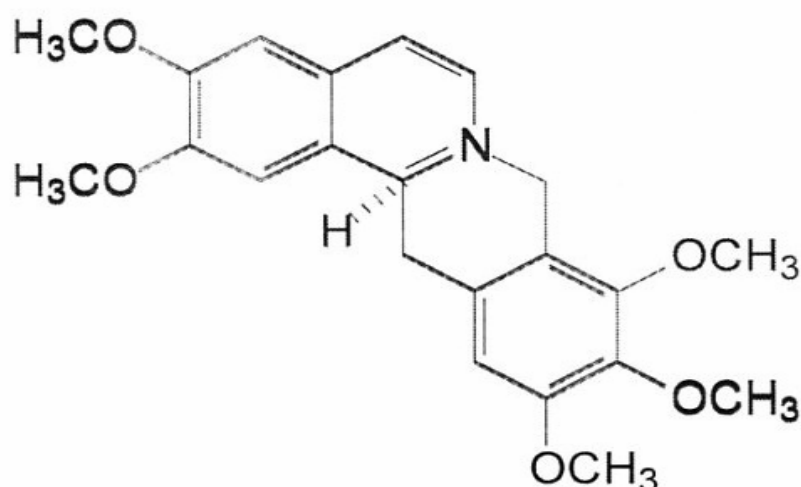


Fig. 6 Superimposed structures of (1)THP and papaverine.

Anyway, the rigid structure of THP would not let to their molecules a proper mobility around its axis such as those of papaverine. This could be the reason why THP was three times less active than papaverine when comparing the relationship among the ED50s, as in average the ED50 of THP was approximately three times greater than that of papaverine, namely 5.5×10^{-5} M/L and 1.8×10^{-5} M/L respectively. ($5.5/1.8 \approx 3$)

On the other hand, while having both drugs a sedative action on the central nervous system, chlorpromazine exhibited an antagonist pattern very different to THP, with a higher affinity for specific receptors. (Fig. 3) The fact that THP and chlorpromazine poses a different action spectrum on pharmacological receptors may have a particular interest because the collateral effects of chlorpromazine, which limit its employment in therapeutics, are mainly due to its blocking actions on several autonomic and central receptors. Such being the case, it seems reasonable to expect that the collateral effects of THP will be different and less severe than those of chlorpromazine, as papaverine is unspecific and less toxic than the former.(18) This may provide an incentive to continue the pharmacological evaluation of THP, with a view to its usage in human beings on account of its potential value as agent acting on the central nervous system.

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