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Original Research

Antagonistic effects of trifluoperazine, imipramine, and chlorpromazine against acetylcholine-induced contractions in isolated rat uterus[1]

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KEY WORDS uterine contraction; acetylcholine; imipramine; pirenzepine; AF-DX 116; 4-DAMP; atropine; chlorpromazine; trifluoperazine

AIM: To examine the effects and affinity of some phenothizines (trifluoperazine, Tri and chlorpromazine, Chl) and antidepressant (imipramine, Imi) drugs on acetylcholine (ACh)-induced uterine contraction. METHODS: Isotonic contractions of rat uterine strips were recorded. ACh was administrated to induce maximal contraction before exchange of nutrient solution. ACh was added 5 min after the testing drugs. The nutrient solution was exchanged 4 times after each agonist (ACh or other agents) to produce maximal contraction. RESULTS: Atropine (Atr, 0.029 - 2.9 μmol $\cdot L^{-1}$), 4-DAMP (3.6 - 360 nmol $\cdot L^{-1}$), pirenzepine (Pir, 0.23 - 23.5 μmol $\cdot L^{-1}$), and AF-DX 116 (0.7 - 35.6 μmol $\cdot L^{-1}$) competitively antagonized the muscular uterine contraction induced by ACh (0.068 - 36068 μmol $\cdot L^{-1}$). The Schild plot was linear ($r = 1.00$). The pK_B and slopes values (95 % confidence limits) were 9.28 ± 0.12 and 1.00 ± 0.10 to Atr, 9.06 ± 0.10 and 1.10 ± 0.08 to 4-DAMP, 7.03 ± 0.15 and 0.99 ± 0.12 to Pir, and 5.60 ± 0.08 and 1.00 ± 0.19 to AF-DX 116. Tri 0.01 - 2 μmol $\cdot L^{-1}$ ($pK_B = 8.39 \pm 0.04$) and Imi 94 - 940 nmol $\cdot L^{-1}$ ($pK_B = 7.21 \pm 0.10$) produced also a competitive antagonism of the muscular uterine contraction induced by ACh ($r = 1.00$), but the slope was only 0.60 ± 0.03 to Tri or 0.83 ± 0.16 to Imi. Chl 2.8 - 5.6 μmol $\cdot L^{-1}$ produced a weak antagonism on amplitude of muscular contraction induced by the cholinomimetic. CONCLUSION: The muscarinic receptors on uterus behaved as M_3 subtype. Tri and Imi, but not Chl, were competitive antagonist of muscarinic receptors of uterus. Imi behaved a simple competitive antagonist at a single site on myometrium, but Tri was not a simple competitive agent at a single site.

The functional and radioligand binding studies have provided evidence for the existence of at least 4 muscarinic receptors subtypes (M_1 - M_4). The classification of the muscarinic receptors is based on their relative sensitivities to antagonists such as pirenzepine (Pir) ($M_1 > M_4 > M_3 \geqslant M_2$), 11[[2-[(diethyl-amino) methyl]-1-piperidinyl] acetyl]-5, 11-dihydro-6*H*-pyrido[2,3-b][1,4]benzodiazepine-6-one (AF-DX 116) ($M_2 \geqslant M_4 \geqslant M_1 > M_3$), and 4-diphenylacetoxy-*N*-methylpiperidine methobromimide (4-DAMP) ($M_3 \geqslant M_4 \geqslant M_1 > M_2$)[1].

Muscarinic agonists cause contraction of uterine smooth muscle, but there is debate about the receptors subtypes involved[1]. While biochemical studies point to M_2 and/or M_4 receptors on rabbit uterus[2], functional studies suggest M_2 and/or M_3 receptors involved on muscular contraction of guinea pig uterus[3]. The different results observed in such studies could be due to not using the better pharmacological tools like Pir, methoctramine (Met, *N*, *N'*-bis{6-[(2-methoxybenzil) amino] hexyl}-1, 8-octanediaminine tetrahydrochloride), 4-DAMP, and himbacine (Him).

Phenothiazines and some antidepressant drugs which exhibit antimuscarinic properties are compounds structurally similar to Pir, AF-DX 116, 4-DAMP or Him[1,4]. Thus, the present study was undertaken to examine the effects and the affinity of some phenothizines and antidepressant drugs on the acetylcholine (ACh)-induced uterine contraction.

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MATERIALS AND METHODS

Drugs Atropine sulfate (Atr, Merck), chlorpromazine dichloridrate (Chl), imipramine chloridrate (Imi), pirenzepine dihydrochloridrate (Pir), and acethylcholine chloridrate (ACh, Sigma), trifluoperazine chloridrate (Tri, SKF-Enila), 4-DAMP methiodide, and methoctramine chloridrate (Met) were kindly supplied by Prof Dr Melchiore (Universita' degli Studi di Bologna). AF-DX 116 (AF-DX) was kindly supplied by Dr Karl Thomae GmbH.

Tissue preparation Wistar rats housed at 20 – 24 ℃ and illuminated from 6:00 to 18:00 were injected im estradiol benzoate 0.5 mg·kg^{-1}. After 3 d, uterine strips, 15 mm long and free from adhering tissues, were suspended in 15 mL of De Jalon's solution (NaCl 154; KCl 5.6; $CaCl_2$ 0.3; $MgCl_2$ 1.4; $NaHCO_3$ 1.7, and glucose 5.5 mmol·L^{-1}) at 37 ℃, and bubbled with air. Isotonic contractions were recorded on a smoked drum under a resting load of 1 g.

Experimental design Following an initial equilibration period of 40 – 50 min in normal solution, the drugs were administrated in baths. The different concentrations of ACh were administrated in not cumulative manner but each concentration was allowed to induce maximal contraction before exchange of nutrient solution. The same procedure was followed when the agonist was studied in presence of phenothiazines, antidepressant drugs, or antimuscarinic agents, and ACh was added 5 min later. The solution was exchanged 4 times after each concentration of agonist (ACh or other agents) to produce maximal contraction. The effects were taken as a % of the maximal contraction for each preparation. The unpaired t-test was used for comparison. The slope, pK_B and r were calculated from Schild regression.

RESULTS

Atr 0.029 – 2.9 μmol·L^{-1}, 4-DAMP 3.6 – 360 nmol·L^{-1}, Pir 0.23 – 23.5 μmol·L^{-1}, and AF-DX 0.7 – 35.6 μmol·L^{-1} competitively antagonized the muscular uterine contration induced by ACh 0.068 – 36068 μmol·L^{-1} (Fig 1).

The Schild plot for these data were linear (r = 1.00) and yielded pK_B and slopes values (with 95 % confidence limits) of 9.28 ± 0.12 and 1.00 ± 0.10 for Atr, 9.06 ± 0.10 and 1.10 ± 0.08 to 4-DAMP, 7.03 ± 0.15 and 0.99 ± 0.12 to Pir, and 5.60 ± 0.08 and 1.00 ± 0.19 to AF-DX), respectively (Tab 1).

Tri (0.01 – 2.0 μmol·L^{-1}; pK_B = 8.39 ± 0.04) and Imi (94 – 940 nmol·L^{-1}; pK_B = 7.21 ± 0.10) did not change the maximal effect induced by high concentrations of the ACh, but antagonized the amplitude of muscular contractions induced by low concentrations of the cholinomimetic (r = 1.00). The slope was significantly different from 1.00 (0.60 ± 0.03) to Tri or 0.83 ± 0.16 to Imi. Chl 2.8 – 5.64 μmol·L^{-1} decreased the maximal effect induced by ACh and produced a weak antagonism on amplitude of muscular contraction induced by low concentrations of cholinomimetic (Tab 1, Fig 1).

Tab 1. Apparent pK_B values for antagonistic effect of atropine (Atr), 4-DAMP, trifluoperazine (Tri), imipramine (Imi), pirenzepine (Pir), AF-DX 116, methoctramine (Met), and chlorpromazine (Chl) against acetylcholine-induced contraction in isolated rat uterus. $\bar{x} \pm s$.

	pK_B	Slope	r	Expts
Atr	9.28 ± 0.12	1.08 ± 0.10	0.96	18
4-DAMP	0.96 ± 0.10	1.10 ± 0.08	0.97	12
Tri	8.39 ± 0.04	0.60 ± 0.03	0.99	15
Imi	7.21 ± 0.10	0.83 ± 0.16	0.98	13
Pir	7.03 ± 0.15	0.99 ± 0.12	0.95	12
AF-DX	5.60 ± 0.08	1.00 ± 0.19	0.98	15
Met	5.760 ± 0.020	0.74 ± 0.23	0.82	11
Chl	not competitive	–	–	6

DISCUSSION

The present results confirmed that Atr, 4-DAMP, Pir, and AF-DX, according to the literature[1], are competitive antagonists of uterine muscarinic receptors and demonstrate that the antagonists interact at a single site on myometrium with the following affinity order: 4-DAMP ≫ Pir ≫ AF-DX. Such affinity order indicates that M_3 receptors are involved in the ACh-induced uterine contraction, since M_4 receptors show high affinity for Pir, AF-DX and 4-DAMP while M_2 receptors show high affinity for AF-DX and low affinity for Pir and 4-DAMP[1,5,6]. The present study also showed that Tri and Imi, but not Chl, competitively antagonized the interaction of ACh with myometrial receptors. In contrast, Imi behaved a simple competitive antagonist at a single site on myometrium. The good correlation observed for antagonistic effect of Tri on the ACh-

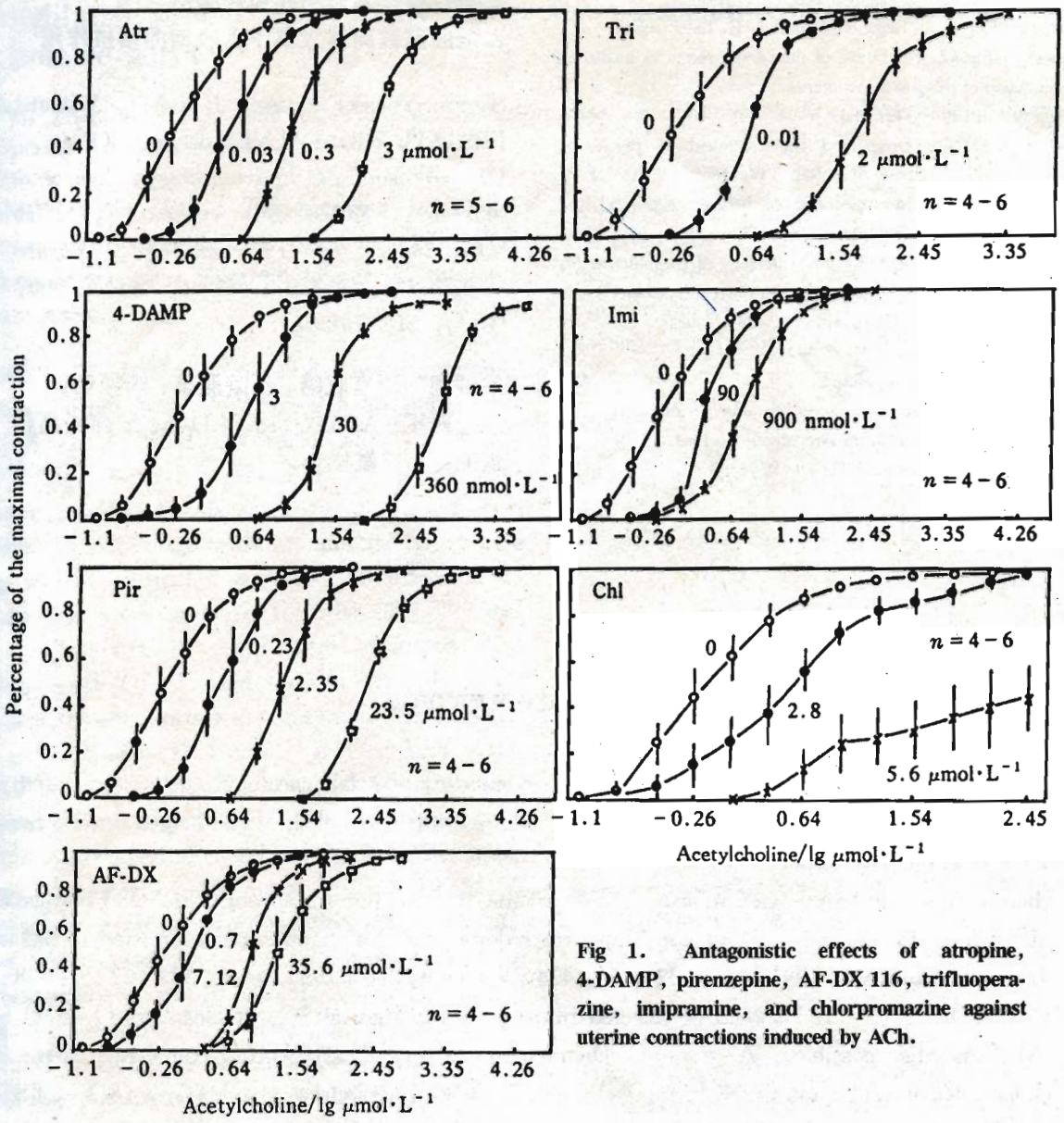

Fig 1. Antagonistic effects of atropine, 4-DAMP, pirenzepine, AF-DX 116, trifluoperazine, imipramine, and chlorpromazine against uterine contractions induced by ACh.

induced contraction (0.99), seems unlikely that the effect results from a simple competitive action at a single site, since the slope obtained from Schild regression analysis was <1. In addition, this finding does not reflect the occurrence of negative cooperatively during the antagonism of ACh-induced contraction since Tri and Chl, which are able to block calmodulin[7], induced different types of antagonism. Thus, it is possible that Tri interacts on muscarinic subtype receptor other than M_3 and/or other sites in myometrium. The insurmountable antagonism observed in studies with Chl may be explained by its inhibitory action on calmodulin[7].

The apparent order of affinity, Atr≥4-DAMP>Tri>Imi≥Pir>AF-DX shows that Tri is better than Imi, Pir, and AF-DX to antagonize the ACh-induced contraction, but its selectivity is uncertain.

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三氟拉嗪、米帕明和氯丙嗪对乙酰胆碱致离体大鼠子宫收缩的拮抗作用[1]

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关键词 子宫收缩; 乙酰胆碱; 米帕明; 哌仑西平; AF-DX 116; 4-DAMP; 阿托品; 氯丙嗪; 三氟拉嗪