

## ANTIMUSCARINIC EFFECTS OF AMITRIPTYLINE HCl AND OPIPRAMOLE HCl IN RAT ILEUM AND RAT DUODENUM

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### SUMMARY

Amitriptyline, a tricyclic antidepressant which is mainly used for major depression treatment, was investigated about its antimuscarinic effects and as a result it was found generally non-selective and 100 fold less active than atropine (1,2,3,4). There are no satisfactory information about opipramole's antimuscarinic activity and selectivity.

Experiments were based on measuring agonist and antagonist responses in isolated tissues in physiological conditions.

### ÖZET

Esas olarak major depresyon tedavisinde kullanılan trisiklik antidepresanlardan amitriptilinin antimuskarinik etkileri araştırılmış ve sonuç olarak atropinden 100 kat az aktif ve genellikle non-selektif bir muskarinik antagonist olduğu düşünülmüştür. Opipramolün antimuskarinik aktivitesi ve selektivitesi açısından yeterli bir çalışma yoktur.

Deneyle izole edilen dokularda, fizyolojik şartlarda agonist ve antagonist cevaplarını ölçülmesi esasına dayanmaktadır.

**Key words:** Antimuscarinic activity, Tricyclic antidepressants, Amitriptyline, Opipramole.

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## INTRODUCTION

After the muscarinic receptors defined, the investigations showed that they consist of different subtypes. The existence of four muscarinic receptor subtypes has been revealed in both functional and radioligand binding studies over the past decade. This evaluation mainly based on the availability of rather selective muscarinic antagonists like pirenzepine, himbacine, methoctramine, AQ-RA 741, 4DAMP and p-FHHSID. This concept of subclassification was recently confirmed by molecular cloning experiments which revealed the existence of five muscarinic receptor genes (5,6,7).

One of the first hints on muscarinic receptor diversity was obtained from experiments on the different intensity effects of gallamine. These studies reported gallamine selectivity on atrial muscarinic receptors (6).

During the subtype selective agonist and antagonist finding studies the investigators found HHSID and its p-Floro analogue, they preferentially demonstrated high affinity on  $M_3$  receptor of smooth muscle and glands than  $M_2$  receptors of heart in functional experiments (7,8).

Tricyclic antidepressants exert atropine like effects by blocking muscarinic receptors in peripheral ganglia, thus many side effects are observed during the treatment. Amitriptyline has properties which in general typify the group of drugs known as tricyclic antidepressants. Many side effects of amitriptyline and other tricyclic antidepressants caused by their antimuscarinic actions in mutant doses (3).

In these studies, we investigated amitriptyline and opipramole's muscarinic receptor subtype selectivities and antimuscarinic activities. With these aims, commercially available tricyclic antidepressants amitriptyline HCl and opipramole HCl were used.

## RESULTS AND DISCUSSION

The proportion of muscarinic  $M_2$  receptor outnumber the  $M_3$  population by about 4:1 in rat ileum. But functional studies showed that, the increases in isometric tension are mediated by primarily muscarinic  $M_3$  receptors in this tissue. In order to evaluate  $M_3$  receptor mediated responses we used carbachol ( $10^{-8}$ - $10^{-4}$ M) in rat ileum preparations. It generally produced a maximum contractile response at  $10^{-6}$ M in this tissue.

Rat duodenum possess  $M_1$  receptors in general which mediate the relaxation of the smooth muscle. Thus, we used  $M_1$  selective agent McN-A 343 ( $3 \cdot 10^{-7}$

$7 \cdot 10^{-4}$  M) as a muscarinic agonist. In this tissue it produced maximum relaxant response at  $3 \cdot 10^{-5}$  M.

Amitriptyline produced parallel rightward shifts without causing inhibition on the maximum response of carbachol in rat ileum (Fig. 1). The  $pA_2$  value calculated by Schild analysis for amitriptyline was obtained as  $7,81 \pm 0,18$  in this tissue. However, amitriptyline inhibited the maximum response of McN-A 343 in rat duodenum (Fig. 2)

Opipramole was found as a non-competitive antagonist in rat ileum and duodenum preparations and at all concentrations caused the inhibition of the maximum responses of both antagonists (Fig. 3-4).

Amitriptyline and opipramole caused marked inhibition on maximum responses of muscarinic agonists in rat duodenum, thus they are considered as non-competitive antagonists in this tissue (Fig. 2).

Other investigators reported different results about amitriptyline's muscarinic receptor subtype selectivities. Many of these studies claimed that amitripty-

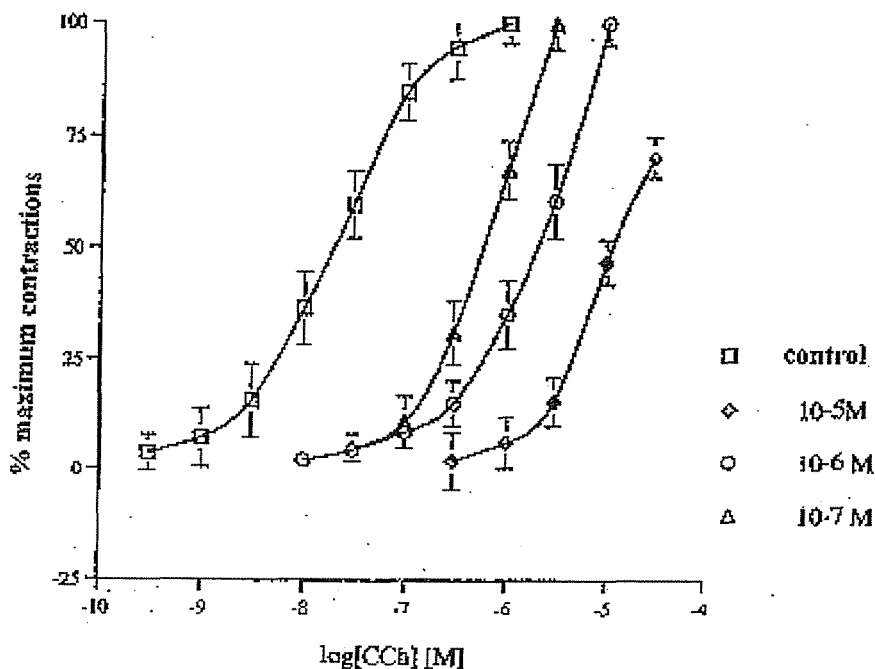


Figure 1: Amitriptyline caused inhibition of the carbachol mediated contractions in rat ileum.

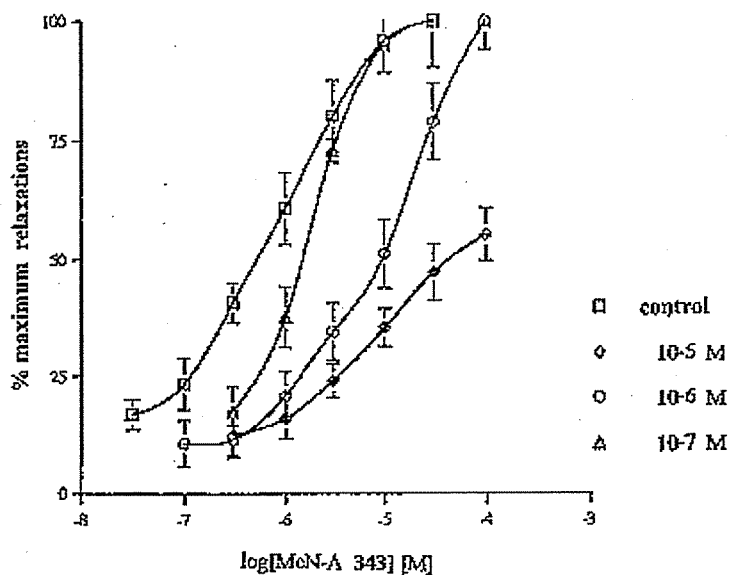


Figure 2: Amitriptyline caused inhibition of the McN-A 343 mediated relaxations in rat duodenum.

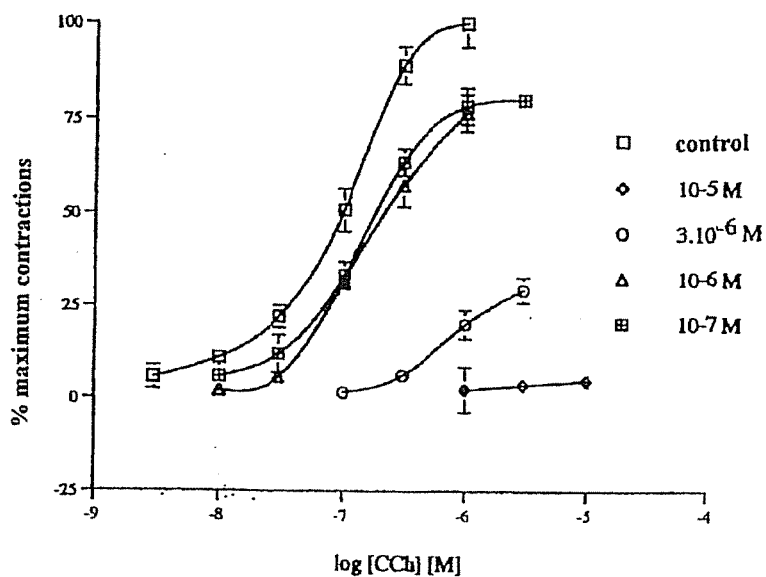


Figure 3: Opipramole caused inhibition of carbachol mediated contractions in rat ileum.

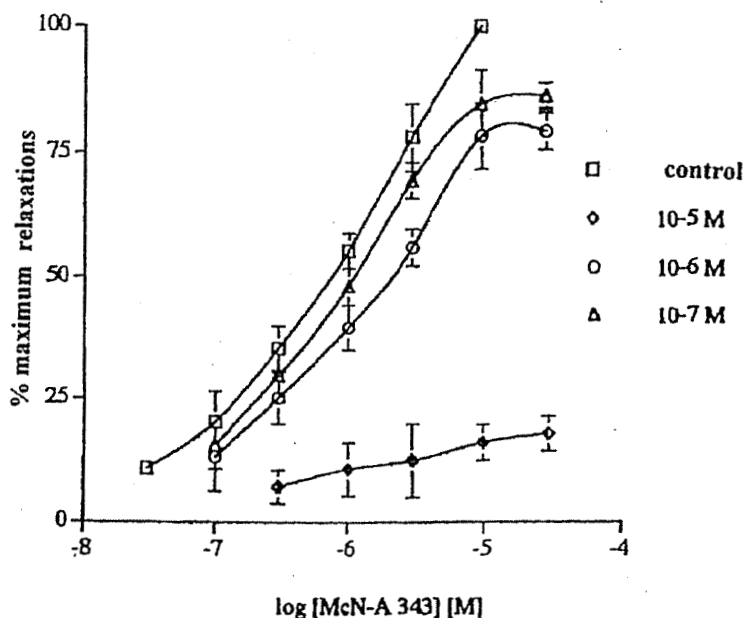


Figure 4: Opipramole caused inhibition of the McN-A 343 mediated relaxations in rad duodenum.

line has no selectivity on muscarinic receptor subtypes. The interaction of amitriptyline with brain muscarinic receptors were assessed using both radioligand and functional assays. The results of radioligand, binding and functional measurements demonstrated that amitriptyline behaved as a non-selective muscarinic antagonist.

On the other hand, the affinity of amitriptyline for muscarinic  $M_1$  receptor was studied in the cerebral cortex and in rabbit vas deferencia utilizing binding studies. The results indicated that amitriptyline exhibits a degree of muscarinic  $M_1$  receptor selectivity (9,10,11).

In our study, amitriptyline and opipramole strongly inhibited carbachol mediated contractions in rat ileum. Amitriptyline produced parallel rightward shifts unlike opipramole in this tissue. Thus, amitriptyline displayed different action in rat ileum, which imply that it possess  $M_3$  muscarinic receptor selectivity. In rat duodenum amitriptyline and opipramole showed potent antimuscarinic activity but they behaved non-competitive.

Finally our findings showed that amitriptyline could have selectivity on muscarinic receptor subtypes different from opipramole.

## EXPERIMENTAL

**Materials:** Force displacement transducer (FT 03), Polygraph (GRASS Model 7PCM 12 C)

**Drugs:** Carbachol, McN-A 343, Amitriptyline HCl, Opipramole HCl.

Carbachol, McN-A 343 and opipramole HCl were dissolved in Tyrode solution whereas amitriptyline HCl in distilled water. Tyrode buffers were made of chemicals of analytical grade purity.

The composition of Tyrode solution was as follows (mM) :

NaCl 137, KCl 2.7, CaCl<sub>2</sub> 1.0 (for duodenum) 1.8 (for ileum), MgCl<sub>2</sub> 1.05, NaH<sub>2</sub>PO<sub>4</sub> 0.42, Glukoz 5.6

**Animals:** Wistar albino rats of either sex (200-300g) were used.

**Methods:** Wistar albino rats were killed by cervical dislocation after ether anesthesia. All tissues were excised, dissected free of connective tissues. Rat ileum and rat duodenum segments were isolated approximately 1.5-2 cm in length. Tissues were suspended between parallel platinum electrodes in Tyrode solution.

All preparations were allowed 30 minutes for equilibration in Tyrode buffer under 500mg 1.5g of resting tensions for rat ileum and duodenum, respectively. Afterwards, concentration-response curves to carbachol in rat ileum and McN-A 343 in rat duodenum was constructed. Tissues were washed at 10 minutes intervals.

In rat ileum the concentration-response curve to carbachol was established in the absence and presence of amitriptyline and opipramole.

Similarly, in rat duodenum the concentration-response curve to McN-A 343 was established in the absence and presence of amitriptyline and opipramole. In these experiments agonist doses were given in non-cumulative manner in order to obtain proper relaxant responses.

Three tissues were used for one concentration of each antagonist and four antagonist concentrations were used in each pA<sub>2</sub> analysis. pA<sub>2</sub> values were determined by Schild analysis method.

Responses were measured as gram changes in isometric tension and calculated as a percentage of the maximum agonist response attained in the initial concentration-response curve.

Agonist and antagonist affinities were calculated, all statistically significant differences were determined by Student's t test.

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