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# MUSCARINIC RECEPTOR SUBTYPES IN HUMAN AND RAT COLON SMOOTH MUSCLE

AURELIO GÓMEZ,* FRANCISCO MARTOS, INMACULADA BELLIDO, ELISABEL MARQUEZ, ANTONIO J. GARCIA, JOSÉ PAVIA and FELIPE SANCHEZ DE LA CUESTA

Department of Pharmacology, Faculty of Medicine, Málaga University, 29080, Málaga, Spain

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**Abstract**—Muscarinic receptor subtypes in human and rat colon smooth muscle homogenates were characterized with [$^3$H]*N*-methylscopolamine ([$^3$H]NMS) by ligand binding studies. [$^3$H]NMS saturation experiments show the existence of a homogeneous population of non-interacting binding sites with similar affinity ($K_D$ values of 1.38 ± 0.20 nM in human colon smooth muscle and 1.48 ± 0.47 nM in rat colon smooth muscle) and with Hill slopes close to unity in both samples of tissue. However, a significant ($P < 0.01$) increase in muscarinic receptor density ($B_{max}$) is found in human colon (29.9 ± 2.9 fmol/mg protein) compared with rat colon (17.2 ± 1.5 fmol/mg protein). Inhibition of [$^3$H]NMS binding by non-labelled compounds shows the following order in human colon: atropine > AF-DX 116 > pirenzepine. Whereas in rat colon the rank order obtained is atropine > pirenzepine > AF-DX 116. Atropine and pirenzepine bind to a homogeneous population of binding sites, although pirenzepine shows higher affinity to bind to the sites present in rat colon ($K_i$ = 1.08 ± 0.08 μM) than those in human colon ($K_i$ = 1.74 ± 0.02 μM) ($P < 0.05$). Similarly, $IC_{50}$ values obtained in AF-DX 116 competition experiments were significantly different ($P < 0.01$) in human colon ($IC_{50}$ = 1.69 ± 0.37 μM) than in rat colon ($IC_{50}$ = 3.78 ± 0.75 μM). Unlike atropine and pirenzepine, the inhibition of [$^3$H]NMS binding by AF-DX 116 did not yield a simple mass-action binding curve ($n_H < 1$, $P < 0.01$) suggesting the presence of more than one subtype of muscarininc receptor in both species. Computer analysis of these curves with a two binding site model suggests the presence of two populations of receptor. The apparent $K_{i1}$ value for the high affinity binding site is 0.49 ± 0.07 μM for human colon smooth muscle and 0.33 ± 0.05 μM for rat colon smooth muscle. The apparent $K_{i2}$ for the low affinity binding site is 8.01 ± 1.0 μM for human samples and 6.07 ± 1.1 μM for rat samples. These values are close enough to suggest that the first subtype of muscarinic receptor may be considered cardiac (M2) and the second subtype glandular (M3). The relative densities of the receptor subtypes are significantly different for both species. Human colon samples show the major densities of subtype M2, 22.62 ± 1.11 fmol/mg protein, this represents 75.66 ± 3.73% of the total receptors. Rat colon samples show the main proportion of subtype M3, 10.50 ± 1.08 fmol/mg protein which represents 61.06 ± 6.30% of the total receptors. This difference in muscarinic receptor subtype proportions could explain the different affinity patterns shown by pirenzepine and AF-DX 116 in human and rat colon samples.

Heterogeneous populations of muscarinic receptors in smooth muscle of the gastrointestinal tract [1–3], urinary bladder [4], respiratory tract [5–8], ciliary body and vasculature of several animal species [9–11] have been reported. Information about the role of muscarinic receptor subtypes in the contraction and secretion processes of colon smooth muscle could be very valuable. This information could provide a theoretical basis for: (i) techniques of gastrointestinal replacement surgery using intestinal segments, and (ii) treatment of gastrointestinal disorders with specific drugs. However, the anatomy, histology and ultrastructure of the animal smooth muscle widely used in experiments, are not totally similar to those of humans, especially samples obtained from the gastrointestinal tract [12]. It would therefore be desirable to investigate the characteristics of colon muscarinic receptors through the study of human tissue. Unfortunately, neither the characterization of muscarinic receptors nor the function of these receptors in different regions of the human gastrointestinal tract have been well clarified [13–15]. This is probably due to the fact that, for humans, there are only two sources of gastrointestinal tissue available for carrying out quantitative measurements: post-mortem tissue and post-surgical specimens. However, the characteristics of both kinds of tissue are different. The condition of post-mortem tissue depends on the length of time between autopsy and death. It is known that in some humans smooth muscle, such as that of the urinary bladder, the activity of the receptors persists [16], but at present it is not known if the activity of the gastrointestinal tract receptors is equally persistent. We therefore studied the characteristics of muscarinic receptors on biopsic post-surgical tissue which is usually fresh. The purpose of this study is to determine the affinity and density of muscarinic receptors in recently resected human colon tissue using receptor binding methods. The muscarinic cholinergic antagonist [$^3$H]*N*-methylscopolamine ([$^3$H]NMS†) was used. In addition, specimens of rat colon were also studied for comparison purposes.

## MATERIALS AND METHODS

Recently resected samples of human colon from

* Corresponding author: Aurelio Gómez Luque, Departamento de Farmacologia, Facultad de Medicina, Campus de Teatinos s/n, 29080, Málaga, Spain.

† Abbreviations: NMS, *N*-methylscopolamine, HHSiD, hexahydrosiladi-phenidol; 4-DAMP, 4-diphenylacetoxy-*N*-methyl-piperidine.

the distal resection margin of colon cancer partial colostomy were obtained by biopsy. Samples were obtained from 29 male patients aged 41–62 years with colon carcinoma. A healthy section of the colon biopsy was carefully separated from the pathological one under the stereomicroscope at room temperature. This was then immersed in Tris–HCl buffer 20 mM, pH 7.4 at 4°. All tissues were dissected, the mucosal, submucosal and serosal layers were removed to leave the smooth muscle layer. Small fragments of human muscle (about 1 g) were placed without buffer into individual polypropylene tubes and frozen at −80°.

In the comparative animal studies, 30 male Wistar rats (200–250 g, Biocenter, Barcelona) were used. Rat colon samples were extirpated and were then individually washed with ice-cold Tris–HCl buffer. The smooth muscle layer was separated from the mucosal and serosal layers by repeatedly pulling strips of muscle through serrated forceps until only the opaque muscle layer remained. The smooth muscle tissue obtained from rat colon was prepared in the same way.

Human and rat tissue homogenates for the ligand binding assay were prepared by adding 20 mL of $Na^+/Mg^{2+}$ Hepes buffer, pH 7.4 (100 mM NaCl; 10 mM $MgCl_2$; 20 mM Hepes) directly to the tube contents and homogenizing with an Ultra-Turrax run at 70% power setting for 20 sec. This was repeated three times. The tube contents were filtered through four layers of buffer-soaked cheesecloth. Then two additional washes of the tube and rotor with 10 mL of buffer were passed through the same cheesecloth. The protein contentration of the crude homogenate obtained was determined by the method of Lowry *et al.* [17]. The final protein concentration was adjusted to 1.5–2.5 mg/mL with buffer. All binding assays were performed in triplicate with [$^3$H]NMS as radioligand. Incubation volumes for all binding assays were 1 mL. The reaction was started by addition of tissue homogenate and continued for 45 min at 30° in a water bath. Kinetic experiments (see Results) indicate that 15–20 min are sufficient to produce equilibration of the concentrations of [$^3$H]NMS (from 0.5 to 3 nM) in this study. The period of equilibrium persisted for over 60 min. An intermediate time of the equilibrium period was 45 min. The reaction was stopped by the addition of 4 mL of ice-cold Tris–HCl buffer 20 mM, pH 7.4. The contents of the tubes were immediately filtered through Whatman 24 mm GF/B filters under vacuum. The filters were washed with 2 × 4 mL of ice-cold Tris–HCl buffer. Non-specific binding was determined by parallel triplicate assays containing atropine sulphate (1.0 $\mu$M). This concentration was established to define non-specific binding in previous studies. Liquid scintillation counting of the filters was performed in 4 mL of kontrogel (Kontron Ltd) using a Betamatic B Kontron spectrometer with a counting efficiency of 44%.

In saturation binding experiments, nine [$^3$H]NMS concentrations (approximately from 0.1 to 8 nM) were incubated with the membranes in a condition of equilibrium. In competition binding experiments, a single concentration of the radioligand (0.4–0.6 nM) was incubated with the membranes in both

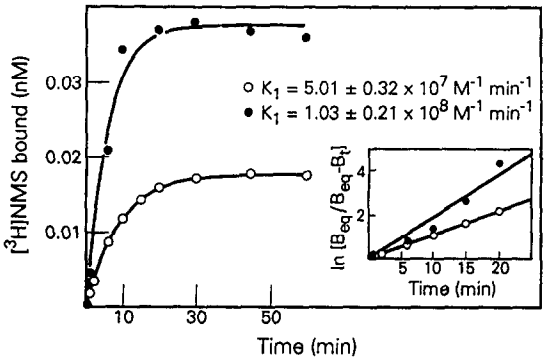


Fig. 1. Association time course of the specific binding of [$^3$H]NMS to human (●) and rat (○) colon smooth muscle homogenates. At indicated times, samples were filtered as described in Materials and Methods. Each point is the mean ± SEM of at least four separate experiments in which [$^3$H]NMS binding for each time point was measured in triplicate. Inset: pseudo-first order plot of the association data, where $B_{eq}$ is the concentration of ligand–receptor complex (fmol/mg protein) at equilibrium and $B_t$ is that at time $t$ (min). The slope of the line is equal to the association rate constants ($K_1$).

absence and presence of competitors. At least 14 concentrations (ranging from 1000 times lower to 1000 times greater than the $IC_{50}$ value for the compound under study) were employed for each compound studied.

The association rate constants ($K_1$) for [$^3$H]NMS binding in both species were calculated from the time course of [$^3$H]NMS association using at least five increased concentrations of radioligand (from 0.5 to 3 nM) to determine their respective association rate constants observed ($K_{obs}$) by the iterative programme KINETIC [18]. Association rate constants ($K_1$ values) were derived from the slope of linear regression obtained by plotting the five $K_1$ observed ($K_{obs}$), against their respective radioligand concentrations [19]. The dissociation rate constants ($K_{-1}$) were determined by incubating samples of human and rat colon homogenates with 0.5 nM of [$^3$H]NMS for 45 min. Unlabelled NMS (10 $\mu$M) was then added and receptor occupancy was measured by filtering at different times (from 1 to 25 min). The dissociation rate constants were calculated [18].

Competition, saturation and kinetic binding data were analysed by using the iterative curve fitting computer package RADLIG [18–20], which includes the following programmes: EBDA (one site model), KINETIC (kinetic data) and LIGAND (two or more site model). The $IC_{50}$ values were corrected to their respective $K_i$ values using the Cheng–Prusoff approximation [21]. All results are the mean and SEM from four to six experiments.

[$^3$H]NMS (sp. act. 79.6 Ci/mmol) was obtained from Amersham (Amersham, UK). Pirenzepine hydrochloride was obtained from Boehringer Ingelheim (Ingelheim, F.R.G.). Atropine sulphate and all other chemicals and reagents used were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). AF-DX 116 (11-[[2-(diethylamino)-

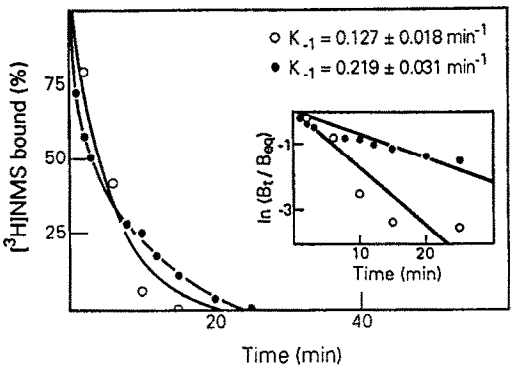


Fig. 2. Dissociation time course of the specific binding of [$^3$H]NMS to human (●) and rat (○) colon smooth muscle homogenates. Dissociation was initiated by the addition of unlabelled NMS (10 $\mu$M) after 45 min of incubation. The receptor occupancy was measured by filtering at indicated times as described in Materials and Methods. Each point is the mean ± SEM of at least four separate experiments in which [$^3$H]NMS binding for each time point was measured in triplicate. Inset: first-order plot of the dissociation data, where $B_t$ represents the concentration of ligand–receptor complex at time $t$ and $B_{eq}$ is the concentration at time zero. The slope of the line is equal to the dissociation rate constants ($K_{-1}$).

ethyl] - 1 - piperidinyl] - acetyl - 5,11 - dihydro - 6*H* - pyrido[2,3,-*b*]1,4,benzodiazepine-6-one) was synthesized by Dr K. Thomae GmbH (Biberach, F.R.G.).

## RESULTS

Figures 1 and 2 show the time course (association and dissociation) of specific binding of [$^3$H]NMS to the colon smooth muscle membranes from human and rat tissue samples. Association experiments show that the specific binding to human and rat colon homogenates is half-maximal after 7–10 and 8–11 min of incubation, respectively. Equilibrium is attained at 15 and 17 min of incubation in human and rat colon samples, respectively. The equilibrium remains for over 1 hr for both tissues. In contrast, the non-specific binding for the two species studied is not time-dependent but remains constant throughout the experiments. It is maximal within 2 min of the beginning of incubation (data not shown). The values of association ($K_1$) and dissociation ($K_{-1}$) rate constants obtained in both species are not different. $K_1$ values are $1.03 \times 10^8 \pm 0.21$ M$^{-1}$ min$^{-1}$ in human colon smooth muscle and $5.01 \times 10^7 \pm 0.32$ M$^{-1}$ min$^{-1}$ in rat colon smooth muscle (Fig. 1). $K_{-1}$ values are 0.21 ± 0.03 and 0.12 ± 0.01 min$^{-1}$ in human and rat samples, respectively (Fig. 2).

The equilibrium dissociation constants ($K_D = K_{-1}/K_1$) for human and rat samples are 2.12 and 2.53 nM, respectively. These dissociation constant values are in the same range as those derived from equilibrium binding studies (Table 1).

Incubation of homogenates in a range of several protein concentrations (from 0.5 to 3 mg/mL) with low concentrations of radioligand (0.4–0.6 nM) under conditions of equilibrium (30°, 45 min) confirms the tissue-linearity of the [$^3$H]NMS binding to human and rat colon homogenates. The amount of both specific and non-specific radioactivity bound is found to increase as a linear function of the protein concentration (data not shown). Under equilibrium conditions, the specific binding of [$^3$H]-NMS in both species is saturable whereas the non-specific binding continues to increase linearly throughout the concentration range (0.2–7 nM) of [$^3$H]NMS studied. Thus, the percentages of specific binding in comparison with the total binding varies from about 83.1 ± 6.5% to 43.7 ± 8.3% in human samples and 79.6 ± 5.2% to 35.9 ± 11.6% in rat samples depending on the concentration of [$^3$H]-NMS present in the incubation medium. The corresponding Scatchard and Hill plots are linear and the slopes of the Hill plots ($n_H$) for both human and rat data are close to unity (Table 1). Consequently, in all experiments and within the concentration range studied, [$^3$H]NMS labels a single, homogeneous population of non-interacting binding sites. Human colon smooth muscle contains a near 2-fold increased population of binding sites with respect to rat colon (Table 1) (Fig. 3).

Competition for [$^3$H]NMS binding by unlabelled

Table 1. Muscarinic receptor density ($B_{max}$), equilibrium dissociation constant ($K_D$) and Hill coefficient ($n_H$) in human and rat colon smooth muscle homogenates derived from [$^3$H]NMS saturation experiments

| Tissue | N | $B_{max}$ (fmol/mg protein) | $K_D$ (nM) | $n_H$ |
|---|---|---|---|---|
| Human colon | 6 | 29.9 ± 2.9 | 1.38 ± 0.20 | 0.97 ± 0.09 |
| Rat colon | 6 | 17.2 ± 1.5* | 1.48 ± 0.47 | 0.98 ± 0.01 |

* Significantly less than human colon (Student's $t$-test) ($P < 0.01$).

Homogenate samples of human and rat colon were incubated with nine different concentrations of [$^3$H]NMS (from 0.1 to 8 nM) for 45 min at 30°.

N, number of specimens.

Each value is the mean ± SEM. Data were obtained by using the programme EBDA.

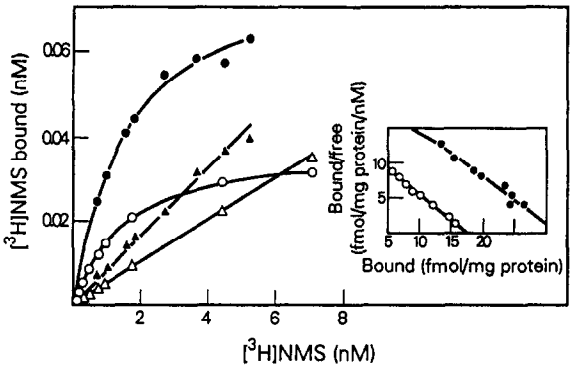


Fig. 3. Representative saturation experiments on [$^3$H]-NMS binding to human colon smooth muscle homogenates (●, total; ▲, non-specific binding) and to rat colon smooth muscle homogenates (○, total; △, non-specific binding). Homogenate samples were incubated at 30° for 45 min with increasing concentrations of [$^3$H]NMS. The inset shows the respective Scatchard plots of specific [$^3$H]NMS binding to human (●) and rat (○) colon homogenates.

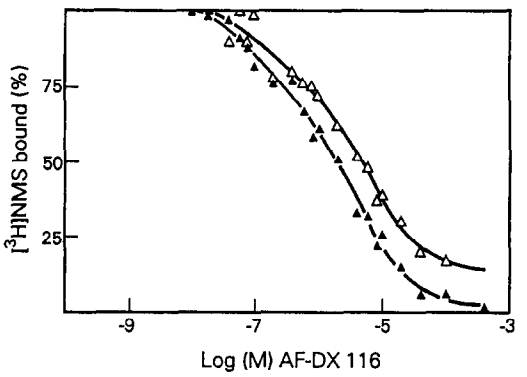


Fig. 5. Competition curves of [$^3$H]NMS (0.4–0.6 nM) binding by AF-DX 116 in human (▲) and rat (△) colon smooth muscle homogenates. Data illustrate a representative experiment performed in triplicate as described in Materials and Methods.

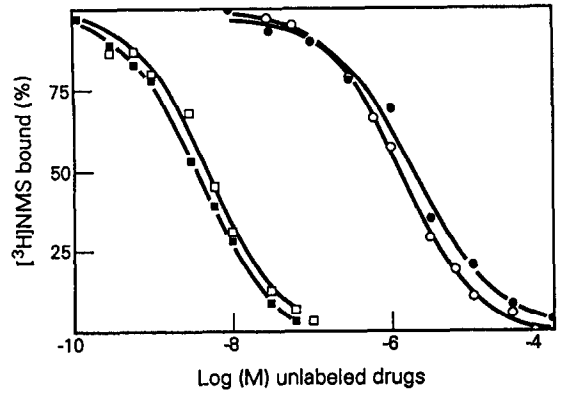


Fig. 4. Competition curves of [$^3$H]NMS (0.4–0.6 nM) binding by atropine and pirenzepine in human colon smooth muscle homogenates (■, atropine; ●, pirenzepine), and rat colon smooth muscle homogenates (□, atropine; ○, pirenzepine). Data illustrate a representative experiment performed in triplicate as described in Materials and Methods.

drugs shows different rank orders of potency in human and rat colon smooth muscle. The rank order of potency in human samples is: atropine > AF-DX 116 > pirenzepine. The rank order of potency is: atropine > pirenzepine > AF-DX 116 in rat samples. Figures 4 and 5 show the displacement curves of pirenzepine, atropine (Fig. 4) and AF-DX 116 (Fig. 5) in human and rat colon smooth muscle. Competition for [$^3$H]NMS binding by the antagonists atropine and pirenzepine produces steep and parallel displacement isotherms that are characterized by Hill coefficients close to unity for both human and rat colon samples. This indicates that these unlabelled antagonists, as well as [$^3$H]-NMS, bind to a virtually homogeneous population of binding sites (Table 2). However pirenzepine, but not atropine, shows a significative ($P < 0.05$) lower affinity to bind to human colon smooth muscle membrane ($K_i = 1.74 \pm 0.02\ \mu M$). In contrast to pirenzepine and atropine, AF-DX 116 binding generates shallow curves with $n_H$ significantly less than unity in both tissues (Table 3). Computer analysis reveals that the data points fit a two site binding model better than a one site model, thus indicating the presence of a mixed population of receptors (Table 3). In human colon membranes AF-DX 116 binds with high affinity (apparent $K_{i_1} = 0.49 \pm 0.07\ \mu M$) to one subpopulation of binding site ($B_{max_1} = 22.62 \pm 1.11$ fmol/mg protein, $75.66 \pm 3.73\%$ of total receptors), and with low affinity (apparent $K_{i_2} = 8.01 \pm 1.0\ \mu M$) to the other subpopulation ($B_{max_2} = 7.33 \pm 1.1$ fmol/mg protein, $24.56 \pm 3.69\%$ of total receptors). In rat colon membranes, just as in human colon, the displacement data are best described by assuming AF-DX 116 to displace [$^3$H]-NMS from two populations of binding site. Unlike in human data, approximately 39% of the sites in rat samples display high affinity for AF-DX 116 ($B_{max_1} = 6.72 \pm 1.07$ fmol/mg protein, $39.07 \pm 6.26\%$ of the total receptors) while the remaining 61% display low affinity ($B_{max_2} = 10.50 \pm 1.08$ fmol/mg protein, $61.06 \pm 6.30\%$ of the total receptors).

## DISCUSSION

The major aim of the present study is to characterize the binding of [$^3$H]NMS to the muscarinic receptors present in human and rat colon smooth muscle. In agreement with studies on other rat tissues, [$^3$H]NMS binding to rat colon homogenates appears to consist of a single population of binding sites. In addition, the calculations of Hill coefficients show neither positive

Table 2. Binding parameters, inhibitory constant ($K_i$) and Hill coefficients ($n_H$) of the inhibition by atropine and pirenzepine of [$^3$H]NMS (0.4–0.6 nM) specific binding to human and rat smooth muscle homogenates

| | Muscarinic antagonist | | | | | |
|---|---|---|---|---|---|---|
| | Atropine | | | Pirenzepine | | |
| Tissue | N | $K_i$ (nM) | $N_H$ | N | $K_i$ ($\mu$M) | $N_H$ |
| Human colon | 6 | 3.50 ± 0.20 | 0.97 ± 0.08 | 6 | 1.74 ± 0.02* | 0.99 ± 0.08 |
| Rat colon | 6 | 2.63 ± 0.38 | 0.92 ± 0.07 | 6 | 1.08 ± 0.08 | 1.03 ± 0.04 |

* Significantly different rat colon (Student's $t$-test) ($P < 0.05$).

$K_i$ values were calculated using the Cheng–Prusoff correction: $K_i = IC_{50}/(1 + L/K_D)$, where $IC_{50}$ was the muscarinic antagonist concentration which decreased the specific binding of radioligand at 50%; $K_D$ was the equilibrium dissociation constant of [$^3$H]NMS determined in both species by saturation experiments; $L$ was the [$^3$H]NMS concentration used (0.4–0.6 nM).

N, number of specimens.

Each value is the mean ± SEM. Data were obtained by using the programme EBDA.

Table 3. Binding parameters of the inhibition by AF-DX 116 of [$^3$H]NMS (0.4–0.6 nM) specific binding to human and rat colon smooth muscle homogenates

| Tissue | N | $IC_{50}$ ($\mu$M) | $n_H$ | Apparent $K_{i_1}$ ($\mu$M) | Apparent $K_{i_2}$ ($\mu$M) | $B_{max_1}$ (fmol/mg protein) ($B_{max_1}$%) | $B_{max_2}$ (fmol/mg protein) |
|---|---|---|---|---|---|---|---|
| Human colon | 6 | 1.69 ± 0.37* | 0.78 ± 0.05† | 0.49 ± 0.07 | 8.01 ± 1.0 | 22.62 ± 1.11‡ (75.66 ± 3.73) | 7.33 ± 1.1 |
| Rat colon | 4 | 3.78 ± 0.75 | 0.68 ± 0.03† | 0.33 ± 0.05 | 6.07 ± 1.1 | 6.72 ± 1.07§ (39.07 ± 6.26) | 10.50 ± 1.08 |

* Significantly different with respect to rat colon (Student's $t$-test) ($P < 0.05$).

† $n_H$ significantly less than unity (Student's $t$-test) ($P < 0.01$).

‡ Significantly different with respect to $B_{max_2}$ (Student's $t$-test) ($P < 0.01$).

§ Significantly different with respect to $B_{max_2}$ (Student's $t$-test) ($P < 0.05$).

The inhibitory concentration of AF-DX 116 ($IC_{50}$) and the Hill coefficient ($n_H$) were calculated using the programme EBDA. The data for the two binding site model were calculated using the programme LIGAND to obtain their respective $IC_{50}$ values and the maximal receptor density ($B_{max}$), apparent $K_{i_1}$ (apparent affinity to the high affinity site) and apparent $K_{i_2}$ (apparent affinity to the low affinity site) were calculated from their respective $IC_{50}$ values corrected by the Cheng–Prusoff equation. $B_{max_1}$ is also expressed as a percentual value of total binding sites.

N, number of specimens. Data are the means ± SEM.

nor negative cooperation. The $K_D$ and the maximal density of binding sites obtained, 1.48 ± 0.47 nM and 17.2 ± 1.5 fmol/mg protein, respectively, agree with those studies in which [$^3$H]NMS was used to measure binding in different rat tissues containing smooth muscle such as the ileum or stomach [22, 23].

The present results confirm the presence of specific binding sites for [$^3$H]NMS in human colon smooth muscle membrane homogenates. Scatchard and Hill plots suggest the presence of a single population of non-interacting binding sites in the human colon. [$^3$H]NMS affinity for these binding sites (1.38 ± 0.2 nM), is similar ($P > 0.05$) to values found in rat colon samples (1.48 ± 0.47 nM). In human colon samples the binding site density is higher than in those of the rat, these results are qualitatively similar to those shown in other tissues [24, 25]. These results differ from those of Gaginella *et al.* [13], who reported a higher number of [$^3$H]-NMS binding sites in the rat pilorus than in the human one. The biological significance of these observed differences in the maximum number of binding site values is at present unknown.

Atropine, pirenzepine [26] and AF-DX 116 [27] respectively, were used as non-selective, M1-selective and M2-selective muscarinic antagonists in competition experiments against [$^3$H]NMS. All these muscarinic antagonists inhibit the binding of [$^3$H]NMS to the binding sites in homogenates of both human and rat colon. The affinities ($K_i$ values) in both species are consistent with those of a muscarinic receptor. This was deduced because the ranking order of antimuscarinic affinity values for human and rat colon samples, obtained in this study, agree with those obtained from rat and

guinea pig intestinal tissues (colon and ileum) in functional studies [28–31]. Three different pharmacologically identifiable receptors have been proposed: M1 (neuronal tissue), M2 (heart tissue) and M3 (exocrine glands). The classification is based on the binding characteristics of three selective ligands, pirenzepine (M1), AF-DX 116 (M2) and hexahydrosiladiphenidol (HHSiD) (M3) [32–34]. The established ranking order of these ligands is the following: (i) for the M1 receptor subtype: atropine ≈ 4-DAMP (4-diphenylacetoxy-*N*-methyl-piperidine methiodide) ≫ HHSiD > pirenzepine ≫ AF-DX 116; (ii) for the M2 receptor subtype: atropine ≫ 4-DAMP ≫ AF-DX 116 ≫ HHSiD ≫ pirenzepine; and (iii) for the M3 receptor subtype: atropine ≈ 4-DAMP ≫ HHSiD ≫ pirenzepine ≫ AF-DX 116. Although HHSiD was not used in this study, the displacement ranking order of the muscarinic antagonist against [$^3$H]NMS in rat colon membrane (atropine > pirenzepine > AF-DX 116) is similar to that mentioned above for M3 muscarinic receptor subtype. This order agrees with the one reported for inhibition of maximum acetylcholine-induced contraction of guinea pig ileum smooth muscle strips ($pA2$ values) [35]. However the rank order of affinity in human colon smooth muscle homogenates is: atropine > AF-DX 116 > pirenzepine, which agrees with the above mentioned order for the M2 muscarinic receptor subtype.

Differences in the antagonist affinity binding pattern in human and rat muscarinic receptors could be explained by the existence of different proportions of muscarinic subpopulations in both species. AF-DX 116 which recognises one uniform population of muscarinic receptor in human atria ($K_i = 0.11\ \mu M$) and submaxillary salivary glands ($K_i = 3.98\ \mu M$) [36], generates shallow curves for both human and rat colon samples (Fig. 5) suggesting the presence of two populations of muscarinic receptor. The respective apparent $K_i$ values for the two subpopulations (Table 3) when compared with AF-DX 116 $K_i$ values reported for the heart and glands are close enough to suggest that the two muscarinic receptor subtypes found in human smooth muscle membranes may be considered cardiac M2 and glandular M3. This has been reported previously for rat and guinea pig ileum smooth muscle [3, 30, 37, 38], and for trachea and bronchus smooth muscle [7]. The different proportions of M2 and M3 muscarinic receptor subtypes reported in these tissues could possibly be attributed to the different functional role played by the M2 and M3 muscarinic receptor subtypes in smooth muscle contraction [3]. AF-DX 116 recognises a major proportion of the M2 subtype (75.66%) in human colon smooth muscle homogenates, whilst the main proportion detected by this compound in rat colon corresponds to the M3 muscarinic subtype. The comparatively higher proportion of M3 muscarinic receptor subtype found in rat colon samples could explain the higher affinity ($P < 0.05$) shown for pirenzepine to bind to muscarinic receptors present in rat colon ($K_i = 1.08 \pm 0.08\ \mu M$). Human colon samples show a comparatively lower proportion of M3 muscarinic receptor subtype which could explain the lower affinity shown for pirenzepine to bind to muscarinic receptors present in human colon ($K_i = 1.74 \pm 0.02\ \mu M$) (Table 2). As demonstrated in functional [39] and radioligand [28] experiments, pirenzepine shows higher affinity for the M3 muscarinic receptor subtype compared to those for the M2 subtype. Nevertheless, the Hill coefficients obtained from pirenzepine competition experiments in this study are not significantly different from unity in both tissues, suggesting the non-existence of a heterogeneous subpopulation. The narrow range of affinity noted for the binding of pirenzepine to the M2 and M3 muscarinic receptor subpopulations, which has already been well reported [22, 40], could explain this observation.

In conclusion, there are at least two muscarinic receptor subpopulations in human and rat colon smooth muscle similar to the M2 and M3 subtypes. In human colon the more abundant being an M2 receptor and the less abundant an M3 receptor. It would be necessary to carry out further functional and binding experiments with selective antagonists in order to clarify the role of both muscarinic receptor subtypes in contraction and secretion processes.

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