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MUSCARINIC RECEPTOR SUBTYPES IN SMOOTH MUSCLE FROM THE BODY OF HUMAN STOMACH

SLOBODAN M. JANKOVI´C AND DUSAN B. BELESLIN

From the Department of Pharmacology, Medical Faculty, Kragujevac, Serbia, FR Yugoslavia.

ABSTRACT

Up to date, there are four pharmacologically characterized subtypes of muscarinic receptors (M_1, M_2, M_3 and M_4). In our study we have investigated muscarinic receptor subtypes in smooth muscle layers of human stomach. Isolated preparations of longitudinal and circular muscle layers from human stomach were used. Acetylcholine, bethanechol, carbachol, pilocarpine and AHR-602 produced concentration-dependent tonic contractions of isolated preparations of both longitudinal and circular muscle layers. Only pilocarpine increased the amplitude of spontaneous contractions of circular muscle preparations.

Atropine, trihexyphenidyl, pirenzepine, telenzepine, hexocyclium, gallamine and scopolamine butylbromide concentration-dependently blocked tonic contractions of isolated preparations of both circular and longitudinal muscle layers caused by acetylcholine. Pancuronium did not block tonic contractions caused by acetylcholine, while para-fluoro-hexahydro-sila-difenidol (pFHHSiD) produced weak concentration-dependent blockade of tonic contractions caused by acetylcholine in circular muscle preparations only. The most potent antagonists were M_1 selective antagonists: trihexyphenidyl, telenzepine and hexocyclium. These results suggested a predominance of the M_1 muscarinic receptor subtype in smooth muscle of the human stomach.

Keywords: Muscarinic receptor subtypes, stomach, body, human.

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INTRODUCTION

With recent advances in gene mapping and synthesis of a great number of selective muscarinic antagonists, five different muscarinic receptor subtypes designated M_1, M_2, M_3, M_4, and M_5 have been recognized. The first four subtypes were characterized by selective antagonists, while the M_5 subtype was determined only by genetical methods. The most subtype-selective antagonists for the time being are trihexyphenidyl, pirenzepine, telenzepine, hexocyclium (M_1); gallamine, pancuronium, AF-DX 116 (M_2); para-fluoro-hexahydro-sila-difenidol (pFHHSiD) and (S)-p-fluoro-hexbutinol (M_3). There are no known selective antagonists for the M_4 subtype, but pirenzepine has low and sila-hexocyclium high affinity for M_4 receptors.[2] M_1, M_3 and M_5 subtypes of muscarinic receptors activate phospholipase C with subsequent formation of inositol-1,4,5-tris-phosphate as a second messenger and release of Ca^{2+} from the endoplasmic reticulum. M_2 and M_4 subtypes inhibit the enzyme adenyl cyclase, thus decreasing the concentration of cAMP intracellularly.[3,4]

The first experiments on muscarinic receptor subtypes

Correspondence: Dr. Slobodan M. Jankovi´c
Dept. of Pharmacology,
Medical Faculty,
P.O. Box 124,
34000 Kragujevac,
Serbia, FR Yugoslavia
Tel. (381) (34) 64986, Fax. (381) (34) 63090

in the stomach showed a difference between mucosal (high affinity for pirenzepine) and smooth muscle receptors.[5,6] Later on, the heterogeneity of smooth muscle subtypes was proven.[7] Using selective antagonists, the M_3 subtype was discovered on smooth muscle cells in the stomach of several species,[8-10] and it was supposed that M_3 receptors were also predominant in the human stomach.[3] However, experiments on human stomach tissue did not specify the subtypes of muscarinic receptor on smooth muscle layers.[11,12] This study was aimed at investigating the subtypes of muscarinic receptors present in longitudinal and circular muscle layers of the body of human stomach.

MATERIALS AND METHODS

Human stomach preparations

Isolated preparations were taken from 42 patients who were operated at the surgical ward of a general hospital in Kragujevac, FR Yugoslavia, from 1990 to 1992. Patients were between 29 and 69 years old, predominantly males (2:1). Sixteen patients were operated for stomach cancer, 12 patients for duodenal ulcer and 14 patients for gastric ulcer. Patients with stomach cancer underwent total gastrectomy and patients with peptic ulcer disease partial gastrectomy (2/3rds gastric resection). In the operating room, a piece of the anterior gastric wall (2 cm in width and 2 cm in length) was excised from the proximal end of the resected portion next to the greater curvature. If total gastrectomy was performed, the specimen was taken from the same place, at a level 3 cm below the esophagogastric junction. The specimen was immediately rinsed in Tyrode solution (NaCl 137 mM, KCl 2.68 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1.0 mM, NaH_2PO_4 0.417 mM, $NaHCO_3$ 11.9 mM and glucose 5.5 mM per liter of solution). About 20 minutes later in the laboratory, the mucosa was removed by sharp dissection. Circular and longitudinal muscle preparations were taken with the long axis of the strip parallel to the muscle layer to be studied. These strips were 1.5 cm in length and 0.5 cm in width, in full thickness and without mucosa. All strips were cut along their longitudinal axis four times (the distance between the two next slices was 1mm) in order to interrupt the muscle layers in different directions. When the isolated preparations had been taken from a patient with gastric cancer, results of the experiment were taken into account only if subsequent histological examination of the isolated preparation proved an absence of malignant infiltration.

The bath and the lever

The strips were mounted in a 15 ml isolated organ bath containing Tyrode solution, aerated continuously with 95% O_2 and 5% CO_2 and maintained at 37°C. One end of the strip was attached to the bath bottom and the other to an isotonic frontal writing lever. Strips were loaded with 1.0g. A magnification of 8:1 was used. Recordings were made on a smoked drum, and vibration was added to the lever holder to reduce the friction of the writing point.

Agonists and antagonists

The strips were allowed to equilibrate for about 45 minutes before any drug was added. Each agonist was added to the bath cumulatively, and isolated preparations were exposed to each concentration of an agonist for 2.5 minutes. After recording the effect of the last concentration of an agonist the bath was drained, washed three times and refilled with fresh Tyrode solution. Between each two agonist cumulations there was a 15 minutes pause which was necessary for recovering the strips. In the studies with blocking agents, the strips were exposed to antagonists for 15 minutes before agonist exposure. In the majority of experiments only one subtype-selective antagonist was tried on each isolated preparation; when it was not possible there was a 1.5 hour pause between the application of two antagonists on the same isolated preparation.

Chemicals

Drugs used in the experiments were; acetylcholine chloride (Sigma Chemical Co., USA), carbamyl-ß-methylcholine chloride (bethanechol chloride, Sigma Chemical Co., USA), carbamylcholine chloride (Sigma Chemical Co., USA), pilocarpine hydrochloride (Pliva, Yugoslavia), AHR-602 (Pharmacological Ins., Yugoslavia), mecamylamine hydrochloride (Sigma Chemical Co.), propranolol hydrochloride (ICN Galenika, Yugoslavia), phentolamine mesylate (Sigma Chemical Co., USA), lidocaine hydrochloride (Alkaloid, FYR Macedonia), cimetidine (Lek, Slovenia), pyrilamine maleate (Sigma Chemical Co., USA), methysergide (Pharmacological Ins., Yugoslavia), nicardipine (Srbolek, Yugoslavia), p-fluoro-hexahydro-sila-difenidol (Research Biochemicals Inc., USA), hexocyclium methylsulphate (Abbot, Italy), pancuronium bromide (Hemofarm DD, Yugoslavia), gallamine triethiodide (May & Baker, UK), telenzepine bihydrochloride (Boeh Ingelheim Pharma GmbH, Austria), pirenzepine dihydrochloride (Boeh Ingelheim Pharma GmbH, Austria), scopolamine butylbromide (Zdravlje, Yugoslavia), trihexyphenidyl hydrochloride (Hemofarm DD, Yugoslavia) and atropine sulphate (Sanofaarma, Yugoslavia).

Statistical analysis

Concentration-response curves were constructed using linear regression according to least squares analysis.[13] The results were considered statistically significant when $P<0.05$. ED_{50} was calculated for each agonist together with its confidence limits (1.96 × standard error).

Effects of antagonists on contractions produced by agonists were evaluated by examining the significance of

agonist concentration-response curve transposition to the right.[13] Level of significance was established at $P<0.05$. The comparison of antagonists was made through their pA_2 values and dissociation constants calculated by "Schild plot" analysis.[14]

RESULTS

Circular muscle strips

A- Spontaneous activity

Only 20% of isolated preparations exhibited spontaneous phasic contractions. The amplitude of spontaneous contractions was between 2 and 6 mm, and frequency between 2 and 4 cycles per minute.

B- Effects of muscarinic agonists

Acetylcholine (from 3.7×10^{-6} M to 6.4×10^{-4}M), bethanechol (from 3.4×10^{-6}M to 6.6×10^{-4}M), carbachol (from 1.4×10^{-8} to 2.5×10^{-7}M) pilocarpine (from 3.2×10^{-7} M to 8.8×10^{-4}M) and AHR - 602 (from 1.0×10^{-5} M to 5.1×10^{-4}M) produced concentration-dependent tonic contractions of isolated preparations of circular muscle ($P<0.01$, $P<0.05$, $P<0.05$, $P<0.05$ and $P<0.05$, respectively) The ED_{50} for acetylcholine, bethanechol, carbachol, pilocarpine and AHR-602 was $2.6\pm0.26\times10^{-5}$ M, $7.9\pm0.3\times10^{-5}$ M, $8.3\pm0.06\times10^{-8}$ M, $2.6\pm0.3\times10^{-5}$ M and $7.2\pm0.3\times10^{-5}$ M, respectively. Maximal contractions were 12.4 ± 1.8 mm, 10.0 ± 1.0 mm, 9.8 ± 0.8mm, 10.2 ± 0.9mm and 9.7 ± 1.0 mm, respectively. Acetylcholine, bethanechol, carbachol and AHR-602 did not affect spontaneous contractions of isolated preparations; pilocarpine increased the amplitude of spontaneous contractions, but this effect was not concentration-dependent ($P>0.05$).

C- Effects of non-muscarinic antagonists

Nicotinic receptor blockers mecamylamine (6.5×10^{-5} M) and hexamethonium (1.22×10^{-4} M) did not affect tonic contractions of isolated preparations caused by acetylcholine ($P>0.05$), while the local anesthetic lidocaine (1.39×10^{-4}M) blocked tonic contractions of isolated preparations caused by acetylcholine ($P<0.05$).

Propranolol (2.3×10^{-5}M) did not affect tonic contractions of isolated preparations caused by acetylcholine ($P>0.05$), but phentolamine (2.10×10^{-5} M) blocked tonic contractions caused by acetylcholine ($P<0.05$).

The H_1 blocker pyrilamine (1.67×10^{-7} M), the H_2 blocker cimetidine (2.64×10^{-6}M) and methysergide (1.42×10^{-6}M) did not affect tonic contractions of isolated preparations caused by acetylcholine ($P>0.05$).

High concentrations of the calcium channel blocker nicardipine (1.29×10^{-8}M and 1.29×10^{-7}M) also blocked tonic contractions of isolated preparations caused by acetylcholine ($P<0.05$).

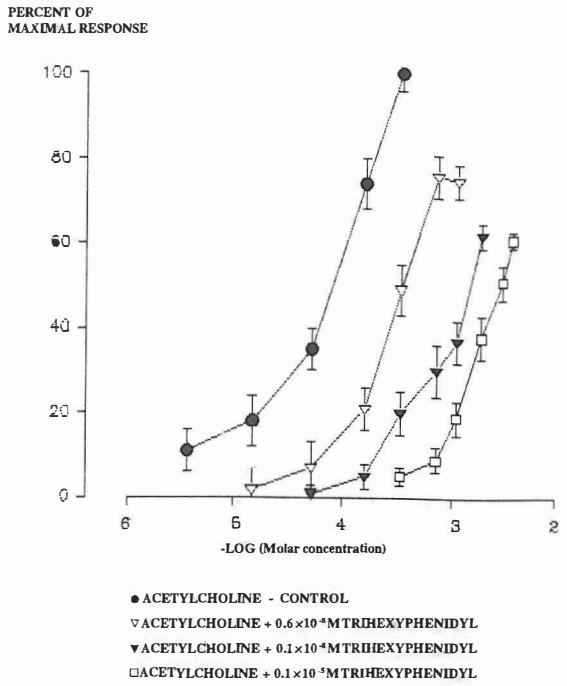

Fig. 1. Effects of trihexyphenidyl on tonic contractions of isolated preparations of longitudinal muscle layer from the body of human stomach. Each point represents the mean response on acetylcholine from four experiments. Error bars represent standard deviations from the mean responses.

D- Effects of muscarinic antagonists

1) Non- selective muscarinic antagonist

Atropine (0.10×10^{-7}M, 0.30×10^{-7}M and 0.10×10^{-6}M) dose-dependently blocked tonic contractions of isolated preparations caused by acetylcholine ($P<0.05$). The pA_2 value for atropine, its dissociation constant and the slope of Schild plot are shown in Table I.

2) M_1- selective muscarinic antagonists

Pirenzepine (1.60×10^{-7}M, 8.0×10^{-7}M and 3.2×10^{-6}M), telenzepine (0.75×10^{-9}M, 3.0×10^{-9}M, 3.0×10^{-8}M and 3.0×10^{-7}M), trihexyphenidyl (0.20×10^{-8}M, 0.60×10^{-8}M, 0.40×10^{-7}M and 1.0×10^{-6}M) and hexocyclium (0.16×10^{-8}M, 0.16×10^{-7}M, 0.8×10^{-7}M and 0.4×10^{-6}M) concentration-dependently blocked tonic contractions of isolated preparations caused by acetylcholine ($P<0.05$). The pA_2 values for pirenzepine. telenzepine. trihexyphenidyl and hexocyclium, their dissociation constants and their slopes of Schild plot are shown in Table I.

3) M_2- selective muscarinic antagonists

Gallamine (0.7×10^{-8}M, 0.7×10^{-7}M and 0.7×10^{-5}M) blocked tonic contractions of isolated preparations caused by acetylcholine ($P<0.05$) in its highest concentration only. The pA_2 value for gallamine, its dissociation constant and the slope of Schild plot are shown in Table I.

Pancuronium (0.9×10^{-5}M) did not affect tonic

Table I. The pA_2 values, dissociation constants and slopes of Schild plot for muscarinic antagonists; circular muscle isolated preparations from human gastric corpus (n = 42 patients).

ANTAGONIST	pA_2 ±CL	Kb	SLOPE ± CL
Atropine	8.91 ± 4.47	1.20×10^{-6}M	-0.75 ± 0.53
Pirenzepine	7.41 ± 4.97	3.89×10^{-8}M	-0.64 ± 3.23
Telenzepine	9.86 ± 0.60	1.38×10^{-10}M	-0.58 ± 0.16
Trihexyphenidyl	9.42 ± 1.79	3.80×10^{-10}M	-0.50 ± 0.50
Hexocyclium	9.92 ± 1.53	1.20×10^{-10}M	-0.53 ± 0.32
Gallamine	5.60 ± 2.7	2.51×10^{-6}M	-0.16 ± 0.26
Pancuronium	did not block tonic contractions caused by acetylcholine		
Scopolamine butylbromide	9.86 ± 0.61	2.09×10^{-10}M	-0.48 ± 0.1
pFHHSiD	5.84 ± 0.06	1.45×10^{-6}M	-0.14 ± 0.02

Table II. The pA_2 values, dissociation constants and slopes of Schild plot for muscarinic antagonists; longitudinal muscle isolated preparations from human gastric corpus (n = 42 patients).

ANTAGONIST	pA_2 ±CL	Kb	SLOPE ± CL
Atropine	8.56 ± 3.35	2.75×10^{-9}M	-1.19 ± 3.50
Pirenzepine	6.25 ± 0.19	5.62×10^{-7}M	-0.66 ± 0.13
Telenzepine	9.73 ± 1.68	4.27×10^{-10}M	-0.76 ± 0.73
Trihexyphenidyl	9.72 ± 1.31	1.91×10^{-10}M	-0.50 ± 0.27
Hexocyclium	9.19 ± 2.53	6.46×10^{-10}M	-0.85 ± 1.15
Gallamine	7.18 ± 1.68	6.61×10^{-8}M	-0.28 ± 0.37
Pancuronium	did not block tonic contractions caused by acetylcholine		
Scopolamine butylbromide	8.67 ± 1.31	2.41×10^{-9}M	-0.55 ± 0.31
pFHHSiD	The blockade was not concentration-dependent		

contractions of isolated preparations caused by acetylcholine (P>0.05).

4) M_3- selective muscarinic antagonists

Scopolamine butylbromide (0.15×10^{-8}M, 0.75×10^{-8}M, 0.75×10^{-7}M and 3.75×10^{-6}M) and p-fluoro-hexahydro-siladifenidol (pFHHSiD: 0.17×10^{-7}M, 0.85×10^{-7}M, 0.17×10^{-5}M and 0.17×10^{-4}M) concentration-dependently blocked tonic contractions of isolated preparations caused by acetylcholine (P<0.05) (Table I) (Fig. 3).

Longitudinal muscle strips

A- Spontaneous activity

Only 10% of isolated preparations exhibited spontaneous phasic contractions. The amplitude of spontaneous contractions was between 3 and 7 mm, and frequency between 3 and 5 cycles per minute.

B- Effects of muscarinic agonists

Acetylcholine (from 3.7×10^{-6}M to 2.7×10^{-4}M), bethanechol (from 0.34×10^{-7}M to 1.51×10^{-5}M), carbachol (from 1.44×10^{-8}M to 2.52×10^{-7}M), pilocarpine(from 0.64×10^{-7}M to 2.36×10^{-4}M) and AHR-602 (from 2.0×10^{-6}M to 10.22×10^{-4}M) produced concentration-dependent tonic contractions of isolated preparations (P<0.01, P<0.05, P<0.01, P<0.05, and P<0.05, respectively). The ED_{50}'s for acetylcholine, bethanechol, carbachol, pilocarpine and AHR-602 were $2.65 \pm 0.26 \times 10^{-5}$M, $0.81 \pm 0.33 \times 10^{-5}$M, $5.33 \pm 0.24 \times 10^{-8}$M, $3.20 \pm 3.17 \times 10^{-6}$M and $9.9 \pm 0.38 \times 10^{-5}$M, respectively. Maximal contractions were 20.5±4.0 mm, 25.3±5.0mm, 33.0±10.5mm, 37.3±7.2mm and 24.0±7.4 mm, respectively. None of these agents affected spontaneous contractions of isolated preparations.

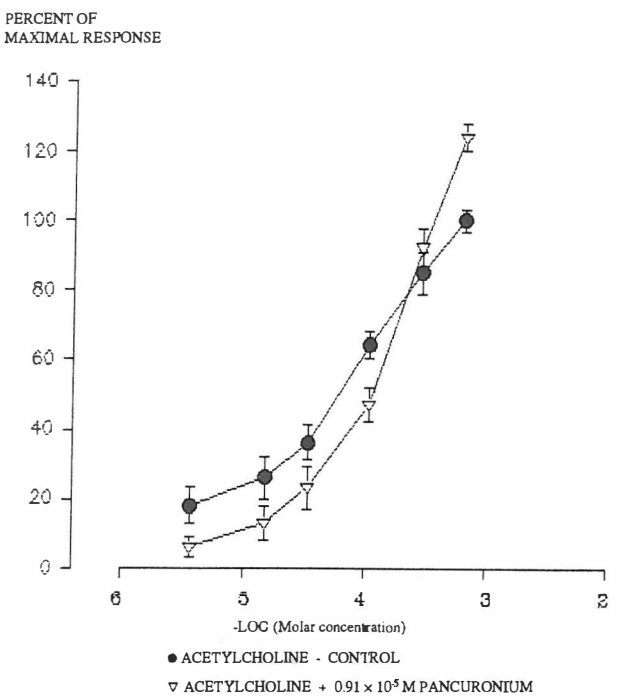

Fig. 2. Effects of pancuronium on tonic contractions of isolated preparations of longitudinal muscle layer from the body of human stomach. Each point represents the mean response on acetylcholine from four experiments. Error bars represent standard deviations from the mean responses.

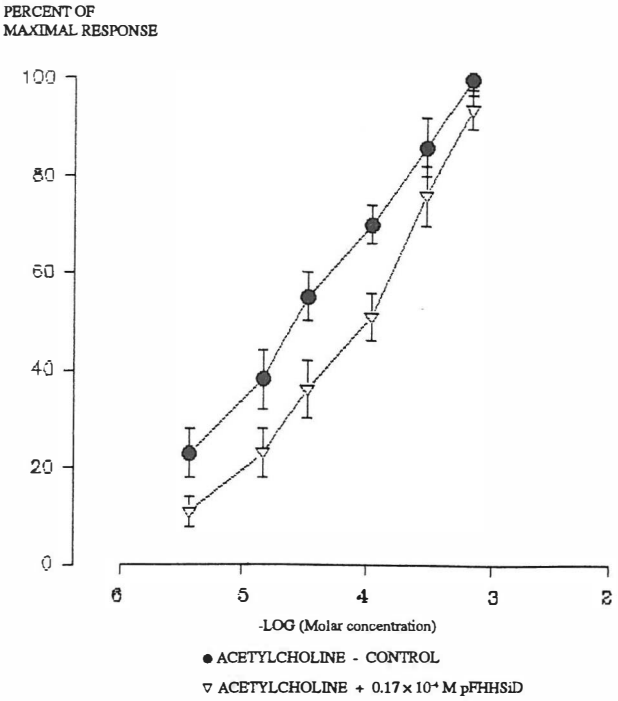

Fig. 3. Effects of pFHHSiD on tonic contractions of isolated preparations of circular muscle layer from the body of human stomach. Each point represents the mean response on acetylcholine from four experiments. Error bars represent standard deviations from the mean responses.

C- Effects of non-muscarinic antagonists

While mecamylamine (6.5×10^{-5}M), the local anesthetic lidocaine (1.39×10^{-4}M phentolamine (2.10×10^{-5}M), propranolol (2.30×10^{-5}M), pyrilamine (1.67×10^{-7}M), cimetidine (2.64×10^{-6}M) and methysergide (1.42×10^{-6}M) did not affect tonic contractions caused by acetylcholine ($P>0.05$), hexamethonium (1.22×10^{-4}M) blocked tonic contractions of isolated preparations caused by acetylcholine ($P<0.01$); only in high concentrations did nicardipine (1.29×10^{-8}M and 1.29×10^{-7}M) block tonic contractions of isolated preparations caused by acetylcholine ($P<0.05$).

D- Effects of muscarinic antagonists

1) Non- selective muscarinic antagonist

Atropine (0.10×10^{-7}M, 0.30×10^{-7}M and 0.10×10^{-6}M) concentration-dependently blocked tonic contractions of isolated preparations caused by acetylcholine ($P<0.05$). The pA_2 value for atropine, its dissociation constant and the slope of Schild plot are shown in Table II.

2) M_1- selective muscarinic antagonists

Pirenzepine (0.16×10^{-6}M, 0.16×10^{-5}M and 0.16×10^{-4}M) dose-dependently blocked tonic contractions of isolated preparations caused by acetylcholine, but only two higher concentrations blocked them significantly ($P<0.05$).

Telenzepine (0.75×10^{-9}M, 0.30×10^{-8}M, 0.30×10^{-7}M and 0.30×10^{-6}M), trihexyphenidyl (0.60×10^{-9}M, 0.60×10^{-8}M, 0.10×10^{-6}M and 0.10×10^{-5}M) and hexocyclium (0.16×10^{-8}M, 0.16×10^{-7}M, 0.8×10^{-7}M and 0.4×10^{-6}M) concentration-dependently blocked tonic contractions of isolated preparations caused by acetylcholine ($P<0.05$) (Table II) (Fig. 1).

3) M_2- selective muscarinic antagonists

Gallamine (0.70×10^{-7}M, 0.70×10^{-6}M and 0.70×10^{-4}M) concentration-dependently blocked tonic contractions of isolated preparations caused by acetylcholine, but only two higher concentrations blocked them significantly ($P<0.05$)(Table II).

Pancuronium (0.91×10^{-5}M) did not affect tonic contractions of isolated preparations caused by acetylcholine ($P>0.05$; Fig. 2).

4) M_3- selective muscarinic antagonists

Scopolamine butylbromide (0.75×10^{-8}M, 0.75×10^{-7}M, 0.75×10^{-6}M and 0.75×10^{-5}M) concentration-dependently blocked tonic contractions of isolated preparations caused by acetylcholine ($P<0.05$)(Table II).

Para-fluoro-hexahydro-sila-difenidol (pFHHSiD: 0.17×10^{-7}M, 0.85×10^{-7}M, 0.17×10^{-5}M and 0.17×10^{-4}M) blocked tonic contractions of isolated preparations caused by acetylcholine only in its two lowest and in its highest

concentration ($P<0.05$). The blockade was weak and not concentration-dependent. Therefore, it was not possible to calculate the pA_2 value for pFHHSiD.

DISCUSSION

Circular muscle layer from the body of human stomach

Only 20% of isolated preparations exhibited spontaneous activity whose frequency corresponded to the frequency of stomach peristaltic waves *in vivo*. Such low percentage of spontaneous activity could be explained by anoxic damage of isolated preparations prior to mounting in the bath. Among used agonists, only pilocarpine affected the spontaneous activity of isolated preparations, but its effect was not concentration-dependent and was therefore inconvenient for detailed analysis.

On the other hand, all muscarinic agonists produced concentration-dependent tonic contractions of isolated preparations. Maximal contractions were not significantly different, but agonists varied in potency. The most potent agonist was carbachol and the least potent AHR-602. These results suggest an absence of the M_2 subtype of muscarinic receptor, but such a conclusion is uncertain owing to the low selectivity of muscarinic agonists.[1]

Nicotinic receptor blockers mecamylamine and hexamethonium did not affect tonic contractions of isolated preparations caused by acetylcholine, but lidocaine produced significant blockade. It is clear that tonic contractions produced by cholinergic agonists in our experiments did not involve nicotinic receptors on ganglionic cells in the gastric wall. Concerning the fact that lidocaine blocks transmission in nerve fibers we could conclude that at least part of the tonic contractions of isolated preparations was a result of activation of muscarinic receptors on nervous structures in the gastric wall.

Propranolol, pyrilamine, cimetidine and methysergide did not affect tonic contractions caused by acetylcholine. This means that acetylcholine acts on specific receptors which are neither adrenergic, histaminergic nor serotoninergic in nature. The weak blockade caused by phentolamine could be explained by its partial agonism on muscarinic receptors.[5] The blocking effect of nicardipine determined that tonic contractions of isolated preparations depend on calcium influx in smooth muscle cells.

Strong and concentration-dependent blockade of tonic contractions by atropine confirmed that acetylcholine had acted on muscarinic receptors. M_1-selective muscarinic antagonists also strongly and dose-dependently blocked tonic contractions caused by acetylcholine. Telenzepine, trihexyphenidyl and hexocyclium were even more potent blockers than atropine, as their pA2 values were higher (Table I). Besides, according to its effects on other tissues, pirenzepine showed great blocking potency. These results suggest a predominance of the M_1 subtype of muscarinic receptors in the circular muscle layer of human gastric corpus.[2]

Gallamine weakly blocked tonic contractions of isolated preparations, only in its highest concentration. The pA2 value for gallamine was very low (5.60). On the other hand, high concentrations of pancuronium did not affect tonic contractions of isolated preparations caused by acetylcholine. Therefore, the presence of the M_2 muscarinic receptor subtype in the circular muscle layer could be excluded.[8]

The M_3-selective muscarinic antagonist pFHHSiD significantly blocked tonic contractions only in high concentrations. The pA_2 value for pFHHSiD was very low (5.84). Considering the high selectivity and potency of this antagonist proved in other tissues, we could conclude that the M_3 subtype was either absent or low-numbered in the circular muscle layer of human stomach. The high pA_2 value for scopolamine butylbromide (Table I) is a result of its low selectivity.

The M_4 subtype is characterized by its low affinity for pirenzepine. Results from our study suggest its absence, but a definite conclusion could not be reached.

Longitudinal muscle layer from the body of human stomach

The incidence of spontaneous contractions was even lower in isolated preparations of longitudinal muscle, i.e., only 10%. Their characteristics (amplitude and frequency), were very similar to those of circular muscle of the human stomach *in vivo*. None of the used muscarinic agonists affected spontaneous contractions of isolated preparations.

All muscarinic agonists produced dose-dependent tonic contractions of isolated preparations. Carbachol and bethanechol were the most potent, suggesting an abundant presence of M_1 and M_3 subtypes of the muscarinic receptor.[9]

Mecamylamine and lidocaine did not affect tonic contractions of isolated preparations. The blocking effect of hexamethonium indicated that acetylcholine had activated excitatory intrinsic neurons in the gastric wall. Knowing that hexamethonium binds ion channels and not the ligand binding area on nicotinic receptors, it is clear that acetylcholine activated the ganglion cells acting on non-nicotinic receptors.

Phentolamine, propranolol, pyrilamine, cimetidine and methysergide did not affect tonic contractions of isolated preparations caused by acetylcholine. Therefore, acetylcholine did not activate adrenergic, histaminergic or serotoninergic receptors. On the other hand, tonic contractions caused by acetylcholine depend on calcium influx in smooth muscle cells of isolated preparations (see effect of nicardipine).

The strong blocking effect of atropine revealed that acetylcholine acts on muscarinic receptors (Table II). The most potent blockers of tonic contractions were telenzepine,

trihexyphenidyl and hexocyclium, all M_1 selective muscarinic antagonists. Pirenzepine exerted weaker blockade compared to circular muscle isolated preparations. The M_1 subtype of muscarinic receptor is predominant in the longitudinal muscle layer of human gastric corpus, as is the case in the circular muscle layer.[1]

While pancuronium did not affect tonic contractions, gallamine exhibited a strong blocking effect on longitudinal compared to circular muscle isolated preparations. This means that the M_2 subtype of muscarinic receptor could be present in the longitudinal muscle layer, but its functional significance is not great. The M_3-selective antagonist pFHHSiD was a bit more efficient on longitudinal than on circular muscle isolated preparations. Nevertheless its effect was not strong enough to consider the M_3 receptors to be present in the longitudinal muscle layer of human gastric corpus in significant numbers. The effect of scopolamine butylbromide was due to its low subtype selectivity.

The low efficacy of pirenzepine implicated the possible presence of M_4- muscarinic receptors, but this could not be concluded without additional experiments with silahexocyclium.

The results of our study suggest the predominance of the M_1 muscarinic receptor subtype in both longitudinal and circular muscle layers of the body of human stomach; other subtypes are either absent or functionally insignificant. This conclusion may have important impact on our choice of drugs for the treatment of motility disorders of the stomach; use of drugs with higher M_1 selectivity could improve the results of the treatment.

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